UNDERSTANDING THE ECOPHYSIOLOGICAL AND BIOMECHANICAL PROPERTIES OF JUVENILE *PINUS RADIATA* IN RESPONSE TO WATER DEFICITS

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ABSTRACT

As the frequency and severity of drought events are expected to increase globally, drought induced reductions in plantation productivity are likely to become more important. This will concern forest managers who wish to improve forest productivity during the establishment and initial growth phases of plantation-grown *Pinus radiata* D. Don. Wood grown during the initial growth phases is in the corewood zone, which in *Pinus radiata* generally has poor wood properties. This restricts its usability, particular for solid timber applications. Therefore, understanding how water deficits impact on patterns of juvenile *P. radiata* productivity and wood variation is important. The objective of this research was to assess how key morphological and physiological processes, carbon fluxes and partitioning, and wood property formation in juvenile *P. radiata* responded to the timing and duration of water deficits.

Using two-year-old *P. radiata* cultivated in a controlled environment, trees were subjected to a well watered control, plus early season cyclical drought, late season cyclical drought and summer drought treatments over the course of one growing season. Needle water potential, tree growth, crown characteristics, biomass partitioning, leaf characteristics, physiological processes and water use efficiency were measured. A carbon balance approach was used to examine how the timing and duration of water deficits modified gross primary production (GPP), net primary production (NPP), carbon fluxes to aboveground net primary production (ANPP) and total belowground carbon flux (TBCF), and the partitioning of GPP to ANPP and TBCF. Wood samples were analysed by X-ray absorption, X-ray diffraction and automated image analysis using Silviscan. Measures of wood density, microfibril angle (MFA), fibre dimensions and modulus of elasticity (*E*) were examined by matching seasonal variation in growth, measured temporally, with variation in wood properties, measured spatially. Further wood samples were assessed for longitudinal shrinkage and acoustic velocity.

Cyclical drought treatments resulted in large fluctuations in needle water potential, while the summer drought treatment resulted in a sustained negative needle water potential over the summer months. Water stress integrals (S_ψ) were 41.4, 66.8, 55.2 and 97.6 MPa-days for the well watered, early season cyclical drought, late season cyclical drought and summer drought treatments, respectively. In general, water deficits decreased tree growth, reduced crown size, reduced biomass accumulation and leaf area, reduced physiological activity and resulted in more enriched values of $\delta^{13}C$, all of which were significantly ($P < 0.05$) affected by treatment. Although the early season drought treatment experienced greater levels of water stress, growth and productivity were superior

to those of the late season drought treatment. Summer drought reduced height, diameter and basal area by 24.7%, 33.1%, and 52.3%, respectively, while aboveground biomass was reduced by 68.3% and total leaf area by 40.0%.

Water deficits substantially decreased fluxes to GPP, NPP, ANPP and TBCF following gradients of tree productivity. Treatment values of GPP were between 1470 and 4142 g C per tree per year. Partitioning of GPP to ANPP and TBCF was not affected by treatment, nor were the F_S /ΔC_R, TBCF/ANPP and ΔC_R/ANPP ratios. Partitioning of GPP was predominantly to TBCF (45 - 56%) for all treatments. Partitioning of soil respiration (F_S) did not significantly differ with treatment but F_S was the dominant component of TBCF (77 - 88%) for all treatments.

Wood properties of juvenile *P. radiata* were sensitive to temporal changes in water availability and associated growth rates. Imposition of seasonal water deficits resulted in higher values of air-dry density and modulus of elasticity (*E*) and decreases in microfibril angle (MFA). These differences were more evident for the late season drought treatment than for the early season drought treatment when compared to the well watered treatment. Late season drought increased density by 10%, *E* by 18% and decreased MFA by 5%, compared with the well watered trees. Seasonal water deficits had no impact on annual average values of density, *E* and MFA in this experiment but significant annual differences existed in cell wall thickness, cell radial diameter and cell populations between the faster and slower growing trees. Well watered trees had higher velocity and also greater longitudinal shrinkage which were significantly different ($P < 0.05$) from the water deficit treatments. Within treatment variation for acoustic velocity and longitudinal shrinkage was greatest for the well watered treatment. Weak to non-existent relationships were observed between longitudinal shrinkage and other wood properties.

The results provide insight for forest managers of *P. radiata* into the importance of managing water deficits to maximise forest production and improve wood quality of juvenile trees. This study demonstrated that late season drought has a more marked impact on absolute growth and wood properties than early season drought, and that water deficits have a greater impact on growth than on partitioning of carbon or ring level wood properties.

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CHAPTER ONE

GENERAL INTRODUCTION

INTRODUCTION

Background

Pinus radiata D. Don productivity is an important issue for forest managers in New Zealand. Increased or diminished growth of *Pinus radiata* is directly related to a site's ability to provide resources to a forest crop. Water supply is one of the most important environmental variables that limit primary production in ecosystems (Axelsson and Axelsson, 1986; Linder, 1987; Johnson, 1990; Gower *at al*., 1992; Osorio *et al*., 1998; Duursma *et al*., 2008). Tree growth and wood formation is a complex process initiated in the tree crown and cambium and is strongly affected by environmental conditions (Antonova and Stasova, 1997; Deslauriers and Morin, 2005; Rossi *et al*., 2006). Water deficits can affect tree and wood growth directly through effects on cambial cells and their derivatives, or indirectly through an effect on photosynthesis and the translocation of assimilates from the crown (Denne and Dodd, 1981; Abe *et al*., 2003; Arend and Fromm, 2007). Radial growth requires an increase in cell volume, which depends on maintenance of high cell turgor pressure (Passioura and Fry, 1992), while cambial activity and wood cell development are strongly dependent on the availability of photoassimilates (Krabel, 2000; Sauter, 2000). Periods of drought affect wood properties by reducing xylem cell production, fibre diameter and cell wall thickness (Whitehead and Jarvis, 1981) and hence, properties such as wood density and microfibril angle may be markedly altered (Drew *et al*., 2009).

In New Zealand, *P. radiata* is likely to be subjected to increasingly erratic seasonal rainfall, more frequent and severe drought, and increased evaporative demand brought about by higher temperatures under predicted climate change in decades to come (Kirschbaum and Fischlin, 1996; Kirschbaum *et al*., 2012). Soil water deficits are common across many eastern regions of New Zealand during summer (Palmer *et al*., 2009) and many of these areas are predicted to receive less rainfall in the future[1] with an increased risk of severe drought (Mullan *et al*., 2005). Forest managers

[1] http://www.mfe.govt.nz/publications/climate/climate-change-effect-impacts-assessments-may08/page4.html

can also influence soil water availability through choices of site, stand density and level of weed control (Nambiar, 1990; Stogsdili *et al*., 1992; Yunusa *et al*., 1995*a*; Watt *et al*., 2003; Watt *et al*., 2006). Furthermore, it has been shown that growth of *P. radiata* is very sensitive to water stress during the juvenile phase (Sands and Nambiar, 1984), and so water deficits are of particular concern to forest managers during establishment and the initial growth phases.

The diminished quality of wood in New Zealand's plantations is an additional concern. The economics of plantation forestry has over time driven the forest industry in New Zealand to increasingly shorter rotations as a result of advances in tree breeding, which have promoted increased growth and changes in silvicultural practices (Downes *et al*., 2000). As a result, the quality of the wood in the *P. radiata* resource has diminished due to an increasing proportion of poor quality corewood, defined as that wood closest to the pith. It is widely acknowledged that wood of the inner most rings of *P. radiata* is inferior for a number of end product purposes (Burdon *et al*., 2004). The corewood zone is characterised by low density, thin cell walls, short fibres with small lumens, high grain angle and high microfibril angle (Macdonald and Hubert, 2002), all traits which contribute to poor stiffness in service and stability during drying. Understanding and improving the corewood zone is of great interest to forest managers and wood processors, and is critical for improving financial returns to both parties.

Our understanding of how trees grow, how management practices and environmental conditions affect wood properties at the fundamental level, and our lack of knowledge as to what can be done to grow faster, straighter, stronger, resin free, stable wood is holding the forest industry back. It is therefore necessary to understand patterns of intra-stem growth and wood properties from stem to stem within a stand of trees. Such information could be used to predict biophysical and financial consequences of forest management decisions. Understanding how the environment, management practices and genetics interact will allow knowledge to be confidently embedded in algorithms and sub-models that predict the growth and quality of trees for use in decision support systems. This level of knowledge will guard against unintended consequences and ultimately increase productivity and wood quality. As such, this study is part of a wider industry effort attempting to address the productivity gap by examining relationships between growth and quality and their drivers.

In response to water deficits, it is important for forest managers to be able to understand growth and canopy dynamics, mechanisms driving carbon partitioning of *P. radiata* plantations, and wood property formation of juvenile *P. radiata*. Moreover, forest managers and researchers need to acquire a better understanding of the environmental drivers of sub-annual variation within a tree ring and the relationship between a rapidly growing cambium and the properties of the wood it

produces. In particular, understanding how drought during the early (spring) and late (autumn) parts of the growing season affect juvenile *P. radiata* is important given the economic importance of the species. The study described here examined patterns of *P. radiata* wood variation within the context of its growth history. By assessing environmental influences on juvenile trees and conducting intensive tree growth and wood property sampling, this study helped increase our understanding of individual tree responses to water deficits with a view to improving end product performance.

Pinus radiata

Pinus radiata is the mainstay of the southern hemisphere plantation softwood trade with approximately 3.9 million hectares planted (Mead, 2013). It currently occupies approximately 1.55 million hectares or 90% of the New Zealand plantation resource (MPI, 2013). The predominance of *P. radiata* in New Zealand can be attributed to numerous factors including its high mean annual increment and versatility for a range of end-uses owing to its ease of drying, treatability and machinability (Cown, 1990). It is suitable for both interior and exterior use, in structural or non-structural applications and can been utilised for timber and plywood, reconstituted into strandboard, particleboard or fibreboard or used in the mechanical or chemical pulp industries. The temperate maritime climate of New Zealand, coupled with its higher level of rainfall throughout the summer, allows *P. radiata* to grow well all year round. In its natural habitat *P. radiata* is adapted to grow in a mild drought prone climate, where the majority of its growth occurs in the wetter winter months (Libby, 1997; Burdon, 2000). The superior growth of *P. radiata* in New Zealand when compared to other species is not due to an increased growth rate, but rather due to its lack of winter dormancy, and its ability to grow throughout the winter if conditions are mild enough. It has displayed a greater adaptability to a range of soil conditions than other introduced species, plus responds well to breeding and silvicultural programs and at present is host to few diseases or pests unlike introduced *Eucalyptus* species (Turner and Lambert, 1986; Burdon, 2000).

Framework for examining tree growth and wood properties in response to water deficits.

In common with other tree species, *P. radiata* exhibits growth which varies systematically during the year and varies in magnitude from year to year (Jackson *et al*., 1976; Mead, 2013). Fundamental to these variations are the environmental factors which affect growth through canopy development, canopy function and partitioning of assimilates (Benson *et al*., 1992). Water deficits are a major constraint imposed on tree growth and anticipated changes in climate, including changes in

precipitation patterns in certain regions on the background of increasing temperatures and atmospheric demand for water, make it imperative to understand *P. radiata* responses to water stress. Although much is known about physiological and morphological responses to water deficits in *P. radiata* (e.g. Shepherd, 1964; Jackson *et al*., 1976; Rook *et al*., 1977; Sheriff and Whitehead, 1984; Snowdon and Waring, 1991; Thompson and Wheeler, 1992), much of this work has had a narrow focus. Furthermore, literature on the effects of early season (spring) and late season (autumn) drought on patterns of growth is very sparse with only Jackson *et al*. (1976) having knowingly examined similar patterns of water deficits in *P. radiata*.

Radial stem growth is a complex process and involves cell division in the cambial zone, followed by cell enlargement, secondary wall thickening and lignification. During the period of cambial activity and tracheid differentiation, the trees and their wood cells are influenced by genetics, its previous history and the environment, which are directly reflected in the developing tree ring (Plomion *et al*., 2001). The environment (climate and nutritional factors, growth regulators, physical stresses) varies continuously over time and consequently, local conditions influencing wood formation at any given time are unique. The distribution of these conditions can be influenced by longitudinal (stem base to apex) or radial (pith to bark) gradients or by factors such as proximity to branches. Any factor which changes the growth pattern of a tree can affect wood properties, and as a result, the variation in wood properties within a stem is large and in seasonal climates, this variation is greatest within an annual ring (Labosky and Ifju, 1972; Downes *et al*., 2008). This study addressed the relationship between the growing cambium as driven by seasonal water deficits and the properties of the wood it produces at the sub-annual level.

Water deficits influence tree growth in a number of different ways. This can be through a decrease in water potential in cambial cells resulting in subsequent inhibition of cell growth, reduction in metabolic activity, and inhibition of stomatal conductance resulting in reduced photosynthetic rate (Centritto *et al*., 2011). Respiration is also affected but to a lesser extent than photosynthesis (Flexas *et al*., 2005). Under severe water stress there is a reduction not only in the amount of foliage produced and the fractional interception of radiation, but also in the efficiency of converting the intercepted energy and the availability of the converted energy as carbohydrates. Cambial activity and xylem cell development are considerable sinks of energy and particularly demanding in sucrose from photosynthesis (Hansen and Beck, 1994; Oribe *et al*., 2003), so the effects of drought on photoassimilates can be manifested in reduced wood and foliage production (Linder, 1987; Arend and Fromm, 2007).

The impact of water deficits on reduced CO_2 assimilation, carbohydrate production, and turgor pressure, is a reduction in rate of cell division and enlargement (Bhattacharjee and Saha,

2014). Previous work has also shown that levels of the growth inhibitor abscisic acid (ABA) increase under drought conditions, contributing to reduced cell expansion (Jenkins and Shepherd, 1974). The adaptation of narrower lumens in the wood cells during times of water deficits means that the cells are much less vulnerable to water stress induced xylem embolism, thus increasing the safety of water conductance (Plomion *et al*., 2001). As a result of reduce cell diameter and thus increased cell population per unit area, density of wood, which is proportional to the amount of cell wall material relative to wood volume, increases. In addition to impacting wood formation, the result of impaired physiological processes is a reduction in physical variables important to commercial forestry such as leaf area, diameter increment and biomass production.

Previous work by Jackson *et al*. (1976) examining the effects of seasonal water deficits on important physical variables in *P. radiata* found that drought imposed during winter/spring reduced height growth considerably during late spring, resulting in an apparent shift of peak growth, while summer/autumn drought virtually eliminated the secondary peak of height increment. Cross-sectional area increment was most strongly reduced by imposed drought during the summer and autumn, and showed marked but over-compensating rehydration responses following removal of drought stress. However, the adverse effects of sustained drought on height increment continued for some time after rewatering. Their study however, examined only a handful of experimental trees, the crowns of droughted trees were not excluded from natural precipitation and the effects of water deficits on tree water status were not directly assessed. Conducting an experiment that builds on that work and addresses those issues has provided a more solid platform for quantifying and understanding how tree growth responds to water deficits throughout the seasons. However, beyond just examining patterns of diameter and height growth, this study has undertaken an integrated approach to assessing tree growth, examining crown and leaf characteristics, biomass partitioning and water use efficiency. This approach has rarely been conducted in studies of *P. radiata* in response to environmental variables.

Quantifying tree growth responses to environmental drivers is essential for predicting patterns of productivity, however, better knowledge of fluxes and partitioning to woody and non-woody tissues in response to water deficits would further enhance our ability to predict patterns of productivity and could provide opportunities to increase forest productivity. Most studies that have examined ecosystem productivity have done so based on aboveground biomass. This is in spite of the fact that aboveground net primary production (ANPP) may account for only 25-30% of gross primary productivity (Giardina *et al*., 2003). A fraction of studies have extended this further by looking at carbon allocation to roots. Such studies have typically shown that water deficits increase the proportion of dry matter partitioned to belowground components at the expense of

aboveground components (Bongarten and Teskey, 1987; Comeau and Kimmins, 1989; Johnson, 1990; Gower *et al*., 1992; Albaugh *et al*., 1998; Coyle and Coleman, 2005), whereas others have found that this proportion does not change or is reversed (Ledig *et al*., 1970; McConnaughay and Coleman, 1999; Espinoza *et al*., 2013). The reason for this disparity may be due to the poor understanding of how water availability influences total carbon budgets. Contradictory evidence may arise because a large proportion of fixed carbon is respired, allocated to mycorrhizae, exuded by roots or released as above- and belowground litter (Ryan, 1991*a*; Giardina *et al*., 2003). In particular, the allocation of carbon belowground has received little attention (Newman *et al*., 2006; Marsden *et al*., 2008) yet carbon loss by respiration from soil is the second largest carbon flux in terrestrial ecosystems, second only to gross primary production (Raich and Schlesinger, 1992). As these components are typically not included in biomass studies, there may be a water deficit effect on carbon partitioning that is not evident from studying biomass alone.

The total net carbon assimilated through photosynthesis over a given time interval is known as gross primary productivity (GPP), while net primary productivity (NPP) represents overall carbon assimilated minus autotrophic respiration (Waring *et al*., 1998). In the past, GPP estimates were difficult to determine because of uncertainties in scaling flux measurements made on small samples over short periods of time and also because of the inherent difficulties in estimating carbon allocation to roots (Ryan, 1991*b*). However, our ability to estimate carbon assimilated through photosynthesis has greatly improved in recent years with the advent of new experimental and modelling techniques (Waring *et al*., 1998). The development of whole carbon budgets have been assisted by a carbon balance protocol developed by Raich and Nadelhoffer (1989) and methods for scaling carbon allocation to roots (Giardina and Ryan, 2002).

Litton *et al*. (2007), analysing published data representing a wide range of planted and natural forests from tropical to temperate climatic regions, found a general pattern of increased carbon partitioned to wood production and less to total belowground carbon fluxes (TBCF) with increasing water supply. In that meta-analysis study, partitioning to aboveground woody components increased with irrigation for all studies, while partitioning to aboveground foliage components increased with irrigation in two studies and decreased in two. Partitioning to TBCF decreased with irrigation for all but one study. Axelsson and Axelsson (1986) also observed increased partitioning to ANPP with increasing water supply in *P. sylvestris*, while Stape *et al*. (2008) observed the same in *Eucalyptus*. These studies have increased our knowledge of carbon budgets and stem wood production, however, their number is limited, highly represented by hardwood species and are exclusively field based and are therefore typically restricted to a control and an irrigated treatment, with little or no control over the intensity and duration of natural precipitation.

As a result, specific information pertaining to the effects of early season (spring) or late season (autumn) drought is very sparse. This study provides the opportunity to quantify the impact of seasonal drought in a highly controlled environment in a softwood species.

To the best of our knowledge, the carbon balance of juvenile *P. radiata* has only been studied by Bown *et al*. (2009) who examined the influence of nitrogen and phosphorus supply and genotype on carbon flux and partitioning. In general, carbon partitioning information pertaining to NPP and respiration in *P. radiata* is limited with only a few published studies (Ryan *et al*., 1996; Bown *et al*., 2009). Furthermore, the use of a carbon balance approach on individual trees has only knowingly been attempted by Vanninen and Mäkelä (2005) and Bown *et al*. (2009). Consequently, this study will provide much needed information towards the literature on juvenile tree responses, *P. radiata* specific responses and individual tree carbon balance responses. In addition, the majority of previous studies examining the effects of water availability on partitioning responses have often failed to directly measure water stress (e.g. Axelsson and Axelsson, 1986; Coyle and Coleman, 2005; Stape *et al*., 2008; Ryan *et al*., 2010). Often, the imposition of water stress over environmental gradients, through imposing absolute or relative amounts of water or based on a water balance, has been the sole determinate of water availability and thus results are poorly related to the water status of the trees under examination.

A positive product of examining short term studies such as that described here and by Bown *et al*. (2009), is that whilst not entirely removing all plasticity resulting from different growth rates, it avoids the bulk of such effects or the effects of ontogenetic drift that arise in the majority of studies that are conducted over many years. Few studies account for the many morphological and physiological changes, including biomass allocation patterns, that occur under the normal course of growth and development over many years before they examine adjustments in biomass allocation in response to fluctuating resource levels (Coleman *et al*., 1994). Another point of contention in the literature is the assumption in many forest carbon budgets that total belowground carbon allocation is partitioned evenly between root production and respiration (e.g. Ruess *et al*., 1996; Binkley and Ryan, 1998; Waring *et al*., 1998; Law *et al*., 2000; Stape *et al.*, 2004; Newman *et al*., 2006). The routine measurement of soil CO_2 efflux in this study will allow us to accurately estimate and model these components to provide a more realistic, functional dataset for analysis and interpretation and demonstrate the effect of seasonal drought on trends of soil CO_2 efflux over one growing season.

The ultimate by-product of allocated carbon to the stem is wood formation, and how it forms, and where is forms within the stem is of importance to forest managers and processors. Within the stem of *P. radiata*, wood is generally referred to as either corewood or outerwood. For ease of definition, corewood is defined as that wood of approximately the first 10 growth rings

(Burdon *et al*., 2004) and juvenile trees are composed entirely of it. Irrespective of this crude definition, the physical and mechanical properties of wood near the pith differs from those of outerwood (DeBell *et al*., 2002) and it is widely accepted that coniferous trees grown in plantation conditions have corewood with physical features that are inferior to those found further from the pith (Butterfield, 2003). The existence of a prominent central stem zone is a common feature of many plantation softwoods and can account for 50% of the stemwood of 25-year-old well thinned, fast grown *P. radiata* (Cown, 1992) (Figure 1.1). The occurrence and importance of corewood in plantation-grown *P. radiata* has been highlighted in a number of wood quality studies (Cown and McConchie, 1983; Tsehaye *et al*., 1995; Cown, 1999; Xu and Walker, 2004). Corewood is generally characterised by low density, thin cell walls, short fibres, high grain angle and high microfibril angle (MFA), with the result that it has low strength and stiffness and poor dimensional stability compared to mature wood (Macdonald and Hubert, 2002).

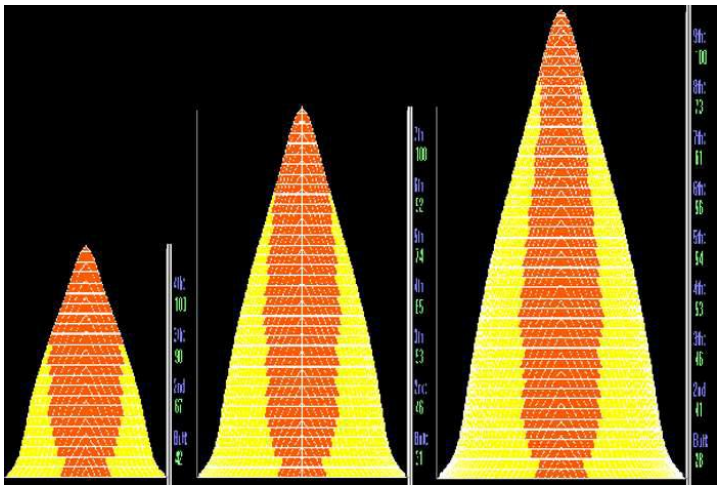


Figure 1.1. Corewood development, from left to right, at tree ages 15, 25 and 35 years. Orange represents corewood, while yellow represents outerwood (*from* Cown, 1999).

Wood stiffness, measured as modulus of elasticity (*E*), and stability are important properties for end-user applications. Modulus of elasticity measures the resistance of wood to deformation under load (Walker and Nakada, 1999) and is often considered more important than strength (modulus of rupture) for predicting wood quality, because *P. radiata* boards rarely break in normal use; much more frequently a load results in excessive deflection (Walford, 1985). Stability is ultimately a measure of differential shrinkage and influences the incidence of bow, crook and twist. The amount of shrinkage is generally proportional to the amount of cell wall material and the amount of moisture removed, and thus shrinkage increases in proportion to wood density. High values of *E* and good stability are essential for solid timber applications and structural wood-based composites and the poor values for these properties found in corewood limits its utilisation and the potential value recovery for processors. For instance, the effect of corewood on structural timber is

poorer grade recovery, lower strength, more distortion and surface checks, and poorer finishing properties (Pearson & Gilmore, 1980). Corewood in structures has also been blamed for excessive movement in response to changing atmospheric conditions (Gorman, 1985).

Modulus of elasticity and shrinkage are controlled and influenced by a variety of wood properties. The properties concerned usually change gradually from the centre of the stem but show different patterns of pith-to-bark variation (Cown, 1992) and it is the poor values of these properties near the pith of the tree that result in poor values for *E* and shrinkage. Microfibril angle and wood density are the two primary wood properties influencing *E* (Cave and Walker, 1994) and shrinkage. Microfibril angle is the angle of the cellulose microfibrils in the cell wall relative to the long axis of the cell. The dominant layer of the cell wall which determines the axial stiffness and the strength of wood is the secondary (S2) layer which occupies about 80% of the volume of the cell wall. Within the first few growth rings, the secondary walls of cells are significantly thinner and the MFA is significantly larger than cells found in outerwood. Within annual growth rings, higher values of MFA are found in earlywood than latewood (McMillin, 1973; Donaldson, 1992). Near the pith, MFA is typically between 30-40 degrees, decreasing to 10-15 degrees at the age of 10-11 years in *P. radiata* (Wu *et al*., 2007), although angles exceeding 60 degrees in corewood have been observed (Butterfield and Pal, 1998). Microfibril angle is inversely related to fibre length in *P. radiata* (Tsehaye *et al.,* 1998) with fibre length increasing from about 1.5 mm close to the pith, to 3.5-4.0 mm in outerwood (Harris and Cown, 1991). Consequently, fibre length is also highly correlated with *E* (Tsehaye *et al*., 1998) and shrinkage. Wood density[2] is affected by a range of fibre characteristics including cell wall thickness and cell diameter, as well as the earlywood to latewood ratio within the annual ring (Cave and Walker, 1994). In corewood, low values of density are associated with thin cell walls, narrow secondary cell layer and large lumen. Density typically increases rapidly over the first 10 to 15 rings from the pith (Xu *et al*., 2004).

The influence of MFA on corewood values of *E* and stability exceeds that of density (Cave and Walker, 1994), which becomes of greater importance as trees mature (Cown *et al*., 1999). This is supported by Baltunis *et al*. (2007) who found a better correlation between MFA and *E* (-0.90) than between density and *E* (0.43) in corewood. The effect of declining MFA from pith to bark is to increase *E* as noted by Cave (1968), who reported a five-fold increase in the stiffness of cell wall tissue of *P. radiata* as MFA decreased from 40 to 10 degrees. Higher MFA in corewood also results in increasing longitudinal shrinkage. Within the cell, the region consisting of non-crystalline cellulose and hemicellulose shrinks in the direction that is orthogonal to the crystalline cellulose microfibrils.

[2] Wood density can be referred to as either "green density" (density of wood when in green condition), "air-dried density" (density at 12% moisture content) or "basic density" (oven-dried density with moisture content of approximately 4%). Distinction between types of wood density will often be made throughout this study.

Therefore, longitudinal shrinkage increases and transverse shrinkage decreases with an increase in the microfibril angle (Megraw *et al*., 1998; Yamashita *et al*., 2009) but in a curvilinear manner (Walker and Butterfield, 1996; Megraw *et al*., 1998). The higher MFA found in the inner corewood means that differential shrinkage in *P. radiata* is mainly a problem in the first three growth rings (Harris and Cown, 1991).

Within an annual ring, both earlywood and latewood are formed reflecting the time of the growing season that the respective cells are formed. When wood formation starts in the spring time (earlywood), the fibres are large with comparatively thin walls, but later in the autumn the cells become smaller and have thicker walls (latewood) (Figure 1.2). The differences in the anatomy of earlywood and latewood fibres result in large within ring wood density differences and good visual distinction of the seasonal growth periods. Basic density of earlywood in *P. radiata* is typically around 350 kg m^{-3}, compared to latewood at 500-600 kg m^{-3} (Cown, 1999). Slower radial growth in latewood results in smaller cells and thicker cell walls and consequently, higher density. Fibre length is approximately 0.5 mm shorter in earlywood than latewood and cell wall thickness is also much less in earlywood compared to latewood (Harris and Cown, 1991). As stems mature, the proportion of latewood increases outwards from the centre, from about 10% at the pith to 50% at maturity (Cown, 1999), driving pith-to-bark increases in density between corewood and outerwood. However, environmental factors such as water deficits can alter the latewood percentage within an annual ring by inducing latewood formation earlier in the growing season (Zahner *et al*., 1964; Whitmore and Zahner, 1966; Brix, 1972; Cregg *et al*., 1988) and thus, significantly influence seasonal or annual values of density.

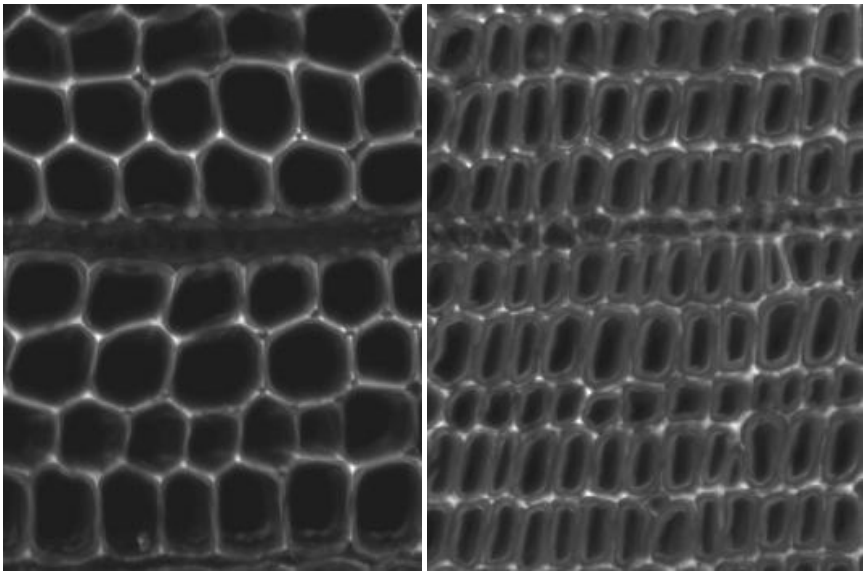


Figure 1.2. Confocal microscope images of earlywood (left) and latewood (right) cells.

Past research has shown that in *P. radiata*, corewood properties can be manipulated by management strategies. Significant gains in important wood properties such as air-dried density, MFA and *E* at single sites have been shown through increasing initial stand density (Lasserre *et al*., 2009), using clones with improved wood properties (Lindstrom *et al*., 2004) and reducing the level of woody weed control (Watt *et al*., 2005). However, Mason (2006) observed in a grass competition experiment that trees with no weed control had the lowest *E*. Significant variation in wood properties has also been shown between sites in New Zealand (Harris, 1965; Cown and McConchie, 1983; Watt *et al*., 2006; Watt and Zoric, 2010).

These studies covering nationwide gradients in environment have shown that temperature is the most important factor in determining wood properties with properties improving in warmer areas and that measures of water availability are of less importance. Harris (1965) surveyed 37 sites and observed that basic density of outerwood was highly correlated with mean annual temperature (r^2 = 0.88), however, the relationship with basic density of corewood was significantly less (r^2 = 0.32), as was the correlation with fibre length (r^2 =0.56), with fibre length differing by about 0.75 mm from the north to the south of New Zealand (Cown, 1999). Having conducted a more extensive survey involving 250 sites throughout New Zealand, Cown and McConchie (1983) found a significant relationship between basic density and mean annual temperature that was better correlated with outerwood (r^2 = 0.49) than with corewood (r^2 = 0.31). There was also evidence that rainfall was positively correlated with wood density, with winter rainfall being particularly important.

In more recent studies examining four-year-old *P. radiata*, Watt *et al*. (2006) found mean annual air temperature and average fractional available volumetric water content, a measure of the root zone water balance, had highly significant ($P < 0.0001$) relationships with *E* (r^2 = 0.53 and 0.33, respectively). However, when examined at the annual level, *E* exhibited a stronger relationship with average minimum temperature (r^2 = 0.57), than either average mean (r^2 = 0.53) or average maximum (r^2 = 0.43) annual temperature. At the monthly level the strongest relationship between average minimum temperature and *E* occurred in March during early autumn (r^2 = 0.60). In another nationwide study (Watt *et al*., 2008), average air temperature and basic density were positively and significantly related (r^2 = 0.34; P = 0.01), however, it was average minimum air temperature during winter that was included in the final multiple regression model for basic density, accounting for 11% of the variance in basic density. In practice, these studies have demonstrated that warmer, wetter sites tend to produce trees with higher than average density.

Although temperature is the dominant driver between sites, experimental studies have shown that water deficits significantly retard tree growth and influence wood properties in juvenile *P. radiata*. Harris *et al*. (1978) found that periodic moisture stress applied to container grown trees

increased earlywood density, mean density and latewood ratio compared with a treatment that had no deficits, although results were not always consistent within annual rings. Fibres were also shorter in trees grown under water stress. Imposed water stress has also been found to reduce measures of acoustic velocity and *E*, have no effect on air-dried density, but increase longitudinal shrinkage, when compared with a well watered control (Nanayakkara *et al*., 2014). Given that acoustic velocity is considered to be a surrogate measure of MFA (Huang *et al*., 2003; Huang and Lambeth, 2007), the large significant differences in velocity between treatments observed in the Nanayakkara *et al*. (2014) study suggest that large differences in MFA existed and that the faster growing well watered treatment had smaller MFA. Whilst such studies have improved our knowledge of the impacts of water deficits on *P. radiata* wood properties, these studies are typically at a low resolution examining annual properties or properties at one point in time and do not quantify the level of water stress imposed on trees as a result of the imposed treatments.

Previous high resolution, sub-annual studies examining the effect of water deficits on wood properties have been predominantly in *Eucalyptus* species (Wimmer *et al*., 2002*a*; Wimmer *et al*., 2002*b*; Drew *et al*., 2009; Drew *et al*., 2011). In the Wimmer *et al*. (2002*a*) study of *Eucalyptus nitens*, lower wood density was formed during the first months of each growing season with an increase later, which is the same trend as observed by Drew *et al*. (2009) in *Eucalyptus globulus*. The effect of well watered conditions was a much more gradual transition from low to high values of air-dried density during a growing season. In trees subjected to cyclic droughts, air-dried density appeared to drop in response to releases from water stress. Irrigated trees in particular formed higher MFA early in the growing season and lower MFA later in the growing season (Wimmer *et al*., 2002*b*). Trees subjected to cyclic droughts showed clear relationships between MFA and soil water deficits, with MFA increasing in response to water stress release. Among treatments, trees subjected to severe drought had the smallest MFA and generally low fluctuations in MFA. Drew *et al*. (2009) found no significant differences in annual values of air-dried density and MFA between rain-fed and irrigated treatments in *Eucalyptus globulus*, however, wood density increased in response to reduced water availability and MFA of faster growing irrigated trees was constantly lower than slower growing trees.

Only one study has knowingly examined the indirect effects of water deficits on high resolution, sub-annual wood properties in *P. radiata* (Watt *et al*., 2005). In this study, the presence and absence of woody weeds created large differences in predawn needle water potential and growth rates over two growing seasons (Watt *et al*., 2003). The result of reduced water availability due to the presence of weeds on juvenile *P. radiata* when averaged across two years was to increase air-dried density (+11%), cell wall thickness (+6%) and *E* (+93%), and significantly reduce MFA (-21%)

and cell radial diameter (-8%), compared with the treatment with better water availability. At the seasonal level, significant water deficits existed for the treatment with weeds during the summer and autumn of the first year and consequently, *E* was doubled, MFA was 10 degrees less, density was over 100 kg m^{-3} greater and cell radial diameter was significantly less. Differences between treatments were less pronounced for density and cell radial diameter during the second season when the water status of both treatments was very similar. However, MFA and *E* were still considerably different.

Given that the effects of water deficits in the Watt *et al*. (2005) study were mediated through the presence and absence of woody weeds, other mechanisms are likely to have contributed to the differences in wood properties between treatments, particularly, during the second season. For instance, the presence of woody weeds would have reduced canopy turbulence and tree sway. Previous research shows that the protection offered from higher plant densities lowers stem deflection through reducing windspeed within the canopy (Raupach, 1992; Green *et al*., 1995) and by damping stem oscillations through increasing the number of collisions with neighbours (Milne, 1991). Increased levels of tree sway have been shown to induce increases in MFA and the second moment of cross-sectional area, and reductions in *E* (Nicholls, 1982; Telewski and Jaffe, 1986; Pruyn *et al*., 2000).

The Watt *et al*. (2005) study also demonstrated how wood properties of juvenile *P. radiata* fluctuate seasonally, irrespective of water deficits. Wood air-dried density reached maximum values during winter and minimum values during spring and summer. There was a strong interaction between time and MFA and *E* for the plots with weeds and water deficits, which was not observed in plots without weeds. In both years for those plots with weeds, values of MFA were highest in spring continuously decreasing to lowest values for the season in mid-winter, similar to that observed by Wimmer *et al*. (2002*b*) in *Eucalyptus nitens*. The trend was reversed for *E* whereby the lowest values were observed in spring of each season before increasing to a winter high. The rate of change was pronounced for *E* which increased six-fold from values of 1.5 GPa in spring of the first year to values of 9.2 GPa during mid-winter at the end of the second year.

The above mentioned high resolution studies have typically demonstrated that density was lower and MFA was higher in faster growing compared to slower growing trees (Downes *et al*., 2004; Watt *et al*., 2005; Drew *et al*., 2009; Drew *et al*., 2011). Linking growth rate with wood properties is a major theme in the literature and has been the focus of numerous studies across many species (e.g. DeBell *et al*., 1994; Zhang, 1995; Mörling, 2002; Wimmer and Downes, 2003; Sarén *et al*., 2004; Bouriaud *et al*., 2005). However, results between studies are often confused and contradictory (Downes and Raymond, 1997). This confusion extends to recent studies of young *P. radiata* which

have produced conflicting information regarding the relationship between growth and wood properties (Watt *et al*., 2005; Mason, 2006; Lasserre *et al*., 2008; Xue *et al*., 2013; Nanayakkara *et al*., 2014). Much has been learned about corewood properties within the last decade, however, our understanding of the processes that lead to trees growing corewood is limited. Radial growth rate is among several theories about factors that drive corewood formation (see Mason (2008) for more detail on competing theories) and whilst the study reported here was not explicitly designed to examine these theories, it will allow for the examination of the relationship between a rapidly growing cambium and the properties of the wood it produces.

Given the wide array of literature on *P. radiata* wood properties, surprisingly little exists where it concerns the effect of water deficits on wood properties. Furthermore, high resolution, sub-annual information is sparse. Specific information regarding the effect of early and late season drought on *P. radiata* is completely unknown and unquantified. It is also important to examine the effects of water deficits independent of other potential epidahic and environmental variables (e.g. wind induced tree sway). Advancing our understanding of the effects of water deficits requires experimentation and sound hypothesis testing and objectives examining the relationship between tree growth and wood properties.

AIM AND SCOPE OF THIS STUDY

Objectives

The aim of this study was to better understand the extent to which the timing and duration of water deficits influence the ecophysiological and biomechanical responses of juvenile *Pinus radiata*. Specifically, this study sought to:

(i) Assess key morphological and physiological processes influenced by water deficits imposed in different seasons,
(ii) Quantify carbon fluxes and partitioning in response to water deficits,
(iii) Investigate the effect of water deficits on wood property formation and patterns, and
(iv) Examine the relationship between tree growth and wood property formation among water deficit treatments.

Hypotheses

A number of working hypotheses were developed and tested in this study. Specifically, these were:

(i) That the timing and duration of water deficits alters rates of photosynthesis impacting on rates of tree growth and canopy development,
(ii) Treatments that promote faster growing trees will allocate more carbon to photosynthetic and structural components at the expense of belowground components,
(iii) That the annual transition of within ring values of microfibril angle, density and modulus of elasticity is heavily regulated through the timing and duration of water deficits, and
(iv) That poor values of wood properties reflect high radial growth rates.

Synopsis of experiment

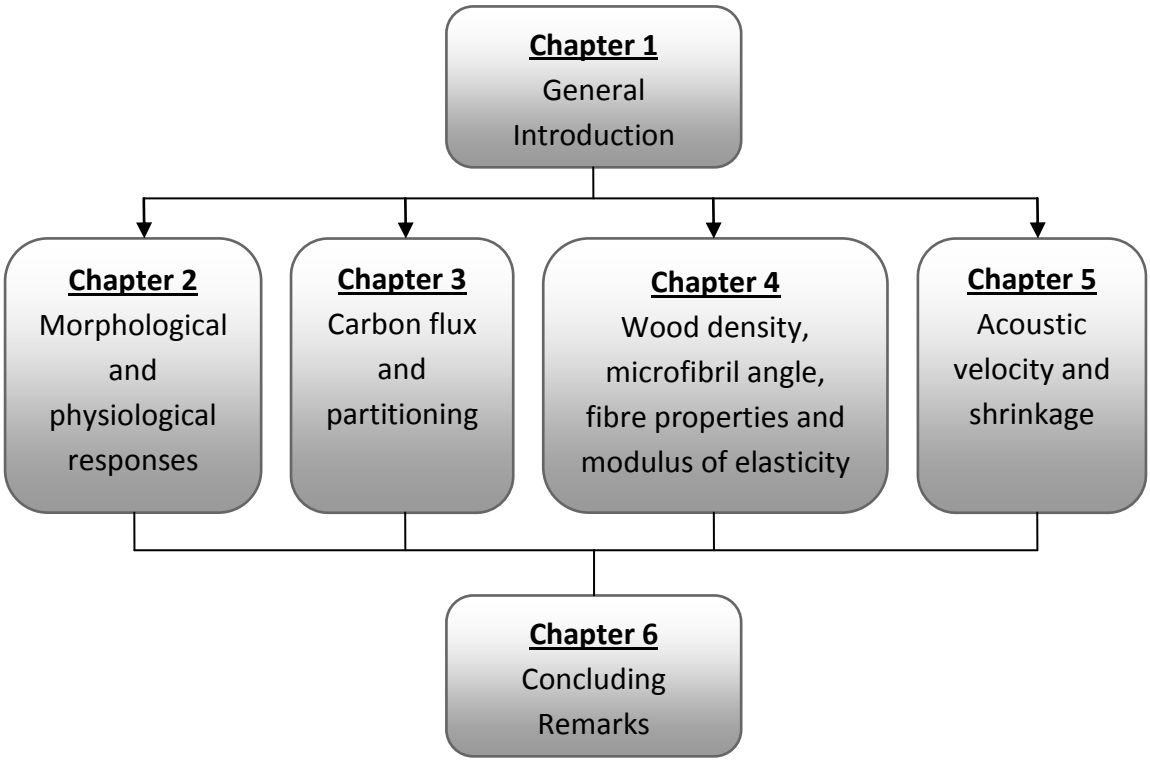
This experiment examined the responses of 96 two-year-old *Pinus radiata* D. Don trees to the timing and duration of water deficits. The tree material used was a high density, high acoustic velocity clone. The experiment was conducted in a water controlled environment for one growing season. The polyhouse environment allowed us to employ water deficit treatments that would have otherwise been unfeasible to emulate in the field. In addition to this, any deleterious and/or adaptive changes displayed by trees in response to water deficits can be synergistically or antagonistically modified by the interaction of other stresses such as wind induced sway when field grown. The treatments were a well watered control, early season cyclical drought (October - January), late season cyclical drought (February - May) and extended summer drought (November - April). The early and late season cyclical drought treatments coincided with expected periods of early- and latewood development.

Extensive resource assessment was conducted and combined to examine the relationship between tree growth and wood property formation among water deficit treatments. Needle water potential and tree dimensions were regularly assessed. Other key morphological and physiological processes were assessed at or towards the end of the experiment, including crown dimensions, biomass partitioning, leaf characteristics, physiological responses and water use efficiency. Absolute carbon fluxes and relative partitioning of above- and belowground components were quantified using a carbon balance approach. The effect of seasonal water deficits on within ring measures of wood density, microfibril angle, fibre dimensions and modulus of elasticity were investigated by matching seasonal variation in growth, measured temporally, with variation in wood properties,

measured spatially within the tree ring. Lastly, measurements of acoustic velocity and longitudinal shrinkage were conducted.

Thesis Structure

This study was designed to undertake a 'whole tree' analysis to quantify the impact that the timing and duration of water deficits has on tree morphology and physiology, carbon fluxes and partitioning and wood property formation. The results of this work are presented in Chapters *Two* (tree growth), *Three* (carbon allocation) and *Four* and *Five* (wood property formation). These chapters were written primarily as stand alone original papers available for submission to a journal and therefore sections of the methodology and results of each chapter are repetitive. Chapter *Six* (Concluding Remarks) summarises key finding of the thesis with emphasis on the relationship between tree growth and wood property formation.



Scope of study

The scope of this study is wide ranging with reference to variables measured and scale. Across all chapters, a wide range of variables were measured including microfibril angle of the S2 layer of the cell wall, water use efficiency, branch length, stomatal conductance, wood density, and carbon

partitioning. These variables encompassed all scales of the tree (Figure 1.3) from molecular ($\delta^{13}C$) through to the whole tree (carbon balance, tree dimensions).

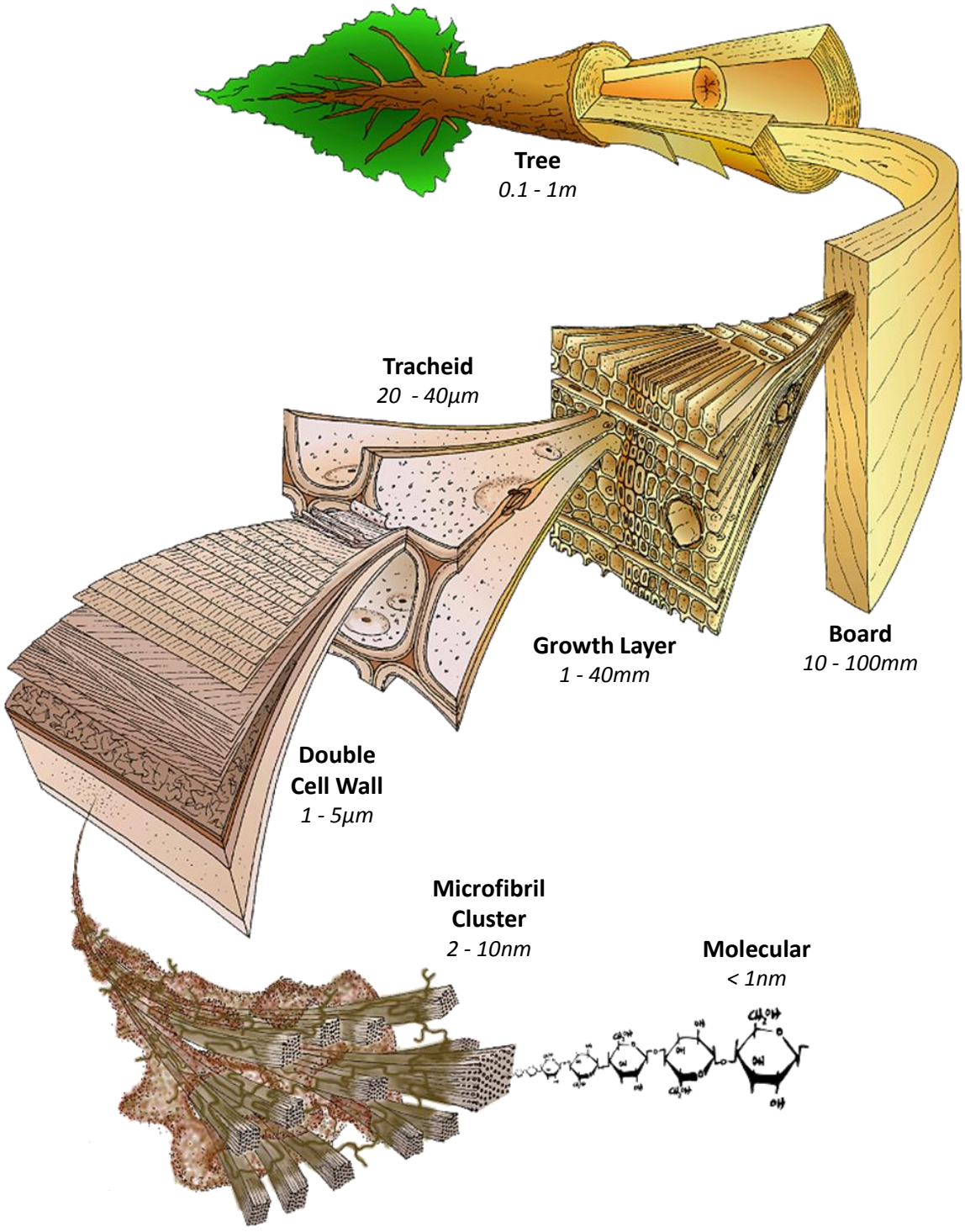


Figure 1.3. A softwood schematic. The dimensions below each of the scale labels are indicative of the size of structural features at that scale (*from* Harrington, 2002).

The main objective of this study was to quantify the response of tree growth and wood properties to water deficits so as to provide the fundamental information currently lacking on the subject. Assumption based hypotheses testing was limited so that potential results were not overlooked or biased against by answering specific questions. Over 850,000 data points were collected and/or calculated and analysed in this study to provide a comprehensive summary of the effects of water deficits on juvenile tree growth and wood formation. The selection of one clone for use within this trial was made to minimise any variability in results due to genetic variation through the use of multiply clones.

CHAPTER TWO

MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF JUVENILE *PINUS RADIATA* TO WATER DEFICITS

SUMMARY

As the frequency and severity of drought events are expected to increase globally (Allen *et al*., 2010), drought induced reductions in plantation productivity are likely to become more important. This will be of concern to forest managers looking to improve forest productivity during the establishment and initial growth phases of plantation grown *Pinus radiata* D. Don. The objective of this experiment was to assess how key morphological and physiological processes in juvenile *Pinus radiata* responded to water deficits. Using two-year-old *P. radiata* cultivated in a controlled environment, needle water potential, tree growth, aboveground biomass partitioning, crown and leaf characteristics, physiological processes and water use efficiency were measured to assess the impact that well watered, early season cyclical drought, late season cyclical drought and summer drought treatments had on productivity. Cyclical drought treatments resulted in large fluctuations in needle water potential, whilst the summer drought treatment resulted in a sustained negative needle water potential over the summer months. The water stress integrals (S_ψ) were 41.4, 66.8, 55.2 and 97.6 MPa-days for the well watered, early season, late season and summer drought treatments, respectively. In general, water deficits decreased tree growth, reduced crown size, reduced biomass accumulation and leaf area, reduced physiological activity and resulted in more enriched values of $\delta^{13}C$, all of which were significantly ($P < 0.05$) affected by treatment. Summer drought reduced height, diameter and basal area by 24.7%, 33.1%, and 52.3%, respectively, while total biomass was reduced by 68.3% and total leaf area by 40.0%, compared with the well watered treatment. The reduction in stem diameter growth was higher for the late season drought treatment than the early season drought treatment when compared with the well watered trees. This suggests that late season drought is more detrimental than early season drought to tree growth. The results provide insight for forest managers of *P. radiata* into the importance of managing water deficits to maximise forest production of juvenile trees.

INTRODUCTION

Pinus radiata D. Don productivity is an important issue for forest managers in New Zealand. Increased or diminished growth of *Pinus radiata* is directly related to a site's ability to provide resources to a forest crop. One of the major factors potentially limiting forest production is water availability (Linder, 1987; Johnson, 1990; Gower *et al*., 1992) and this is of particular importance for juvenile conifer growth (Sands and Nambiar, 1984). Water deficits can greatly influence the amount of foliage produced by a stand and consequently have a direct effect upon the fractional interceptance of radiation (Linder, 1987) and thus, net carbon gain and the potential growth rate of the stand.

During drier periods, reductions in available soil water result in closure of stomata which in turn constraints growth of *P. radiata* and other conifers. While this response to water deficits helps conserve water by limiting evaporative losses, it also reduces carbon dioxide uptake, thereby reducing photosynthetic rate, tree growth and wood production (Waring and Franklin, 1979; Benecke, 1980; Whitehead, 1985; Thompson and Wheeler, 1992; Yunusa *et al*., 1995*b*). Many plant traits are related to water uptake including hydraulic conductance, resistance to embolism, leaf to root ratios and root distribution (Hacke *et al*., 2000; Pinol and Sala, 2000; Martinez-Vilalta *et al*., 2004). Earlier work by Rook *et al*. (1977) showed that greater levels of water stress caused transpiration and photosynthesis to decrease to near zero and older foliage to begin shedding. Furthermore, rates of cell division are depressed and cell expansion is reduced in response to water deficits (Shepherd, 1964; Sheriff and Whitehead, 1984).

With such pronounced physiological responses to water deficits in *P. radiata*, variation in morphology is expected. In response to water deficits, Rook *et al*. (1977) found that diameter and root growth were among the first processes to be affected. Numerous studies of *Pinus* species have investigated growth responses to water availability (Jackson *et al*., 1976; Bongarten and Teskey, 1987; Linder *et al*., 1987; Johnson, 1990; Albaugh *et al*., 1998). As a generalisation, morphological features such as diameter, height, basal area and leaf area are detrimentally impacted by water deficits, which is of concern to forest managers looking to optimise forest growth.

Furthermore, drought induced changes in soil carbon and nutrient availability of forest soils may lead to reduced rates of nutrient uptake by trees. In stands with reduced precipitation and thus reduced decomposition, immobilisation of nutrients in soil organic matter may limit carbon assimilation through inhibition of nutrient uptake from soils (Hanson and Weltzin, 2000). Tree growth is strongly related to foliar nitrogen concentration in conifers, and therefore greater access to water increases nutrient uptake and rates of needle growth (Axelsson and Axelsson, 1986).

Water deficits may also influence juvenile conifer productivity by altering partitioning of biomass between above- and belowground tree components. Previous studies of *P. radiata* subjected to varying degrees of water availability have shown that decreased availability of water reduces aboveground biomass accumulation (Snowdon and Benson, 1992; Woods *et al*., 1992; Yunusa *et al*., 1995*b*; Watt *et al*., 2003; Espinoza *et al*., 2013). However, the partitioning of biomass between aboveground components can be contradictory between studies (Snowdon and Benson, 1992; Watt *et al*., 2003).

Tree water stress can be interpreted from the ratio of the two stable carbon isotopes ^{12}C and ^{13}C found in plant material, known as $\delta^{13}C$. The $\delta^{13}C$ ratio is the result of discrimination against the heavier $^{13}CO_2$ during diffusion through the stomata and subsequent carboxylation in favour of the lighter $^{12}CO_2$ (Farquhar *et al*., 1989; Brienen *et al*., 2011). Discrimination is linearly related to the ratio of leaf intercellular CO_2 concentration (C_i) to atmospheric CO_2 concentration (C_a) (i.e. C_i/C_a), which reflects the balance between the rate of inward CO_2 diffusion, mediated by stomatal conductance, and the rate of CO_2 assimilation in photosynthesis (Warren *et al*., 2001). Hence, drought stress related decreases in stomatal conductance and relative CO_2 concentration in the leaf are often correlated with increases (less negative values) in $\delta^{13}C$ of newly formed photosynthates (Farquhar *et al*., 1989). Water use efficiency (the ratio of assimilation to transpiration) is also related to C_i/C_a, thus the $\delta^{13}C$ ratio and water use efficiency are positively related. The $\delta^{13}C$ signature of tree material is widely regarded as a reliable proxy for water use efficiency (Kruse *et al*., 2012).

Past research has shown that forest managers can influence soil water availability through choices in site, stand density and level of weed control (Nambiar, 1990; Stogsdili *et al*., 1992; Yunusa *et al*., 1995*a*; Watt *et al*., 2003; Watt *et al*., 2006). In addition to the impact of forest management practices on water availability, global climate change is increasingly affecting seasonal rainfall distribution (Basher, 2000; Kenny, 2001). Possible changes in water availability due to changes in weather, with increasingly erratic seasonal rainfall and more frequent and severe drought events, coupled with increased evaporative demand brought about by higher temperatures have the potential to alter forest growth and reduce primary production (Kirschbaum and Fischlin, 1996). At present, soil water deficits are common across many eastern regions of New Zealand during summer (Palmer *et al*., 2009) and many of these areas are predicted to receive less rainfall in the future[3] with an increased risk of severe drought (Mullan *et al*., 2005).

It is important for forest managers to be able to understand the response of growth and canopy dynamics of juvenile *P. radiata* to water deficits. In general, forest managers and researchers

[3] http://www.mfe.govt.nz/publications/climate/climate-change-effect-impacts-assessments-may08/page4.html

have a suitable understanding of the impacts of extended summer drought on accumulative growth and general productivity of *P. radiata* (e.g. Snowdon and Waring, 1991), however, this knowledge does not extend to the consequences of early season (spring) or late season (autumn) drought. Given that morphological and physiological processes can be different between spring and autumn and that different types of wood are being formed (earlywood vs. latewood), it is necessary to examine how early and late season drought compares with extended summer drought and well watered conditions. Consequently, early and late season drought results were of primary interest in this experiment.

The primary objective of this experiment was to quantify how key growth characteristics in juvenile *P. radiata* responded to the timing and duration of water deficits over the course of one growing season. Specifically, this study sought to (i) examine the effect that the timing and duration of water deficits has on productivity, (ii) quantify how early and late season water deficits influences aboveground biomass accumulation and partitioning, and (iii) investigate water use efficiency in response to the timing and duration of water deficits.

METHODS

Experimental location and plant material

The experiment was undertaken in a polyhouse which provided growing conditions where water supply could be controlled. The plant material used was *P. radiata* D. Don clonal stock produced using somatic embryogenesis. This plant material was a high wood density, high acoustic velocity clone (Clone 35, Forest Genetics CellFor Ltd). These characteristics have been shown to be exhibited consistently across a wide environmental range throughout New Zealand. The nursery raised trees were planted in the polyhouse for a two year period during which the experimental treatments were applied during the second year.

Soil medium

The trees were planted in a free draining loamy sand soil within 200 litre pots to reduce any potential for water logging and root binding. Soil texture and particle size were determined using a hydrometer for smaller particles and sieve for larger particles prior to the experiment. Particles were separated into <0.002, 0.002-0.06, 0.06-0.2, 0.2-0.6 and 0.6-2.0 mm size fractions. A comprehensive set of soil chemical measurements were made (Table 2.1). Six bulked soil samples were analysed for

pH in water, total carbon (C), total nitrogen (N), Olsen phosphorus (P), exchangeable bases and cation exchange capacity (CEC), following the methods described by Blakemore *et al*. (1987).

Table 2.1. Soil physical and chemical properties.

Variable	Value
Soil physical properties (%)	
Coarse sand	59
Medium sand	17
Fine sand	8
Silt	8
Clay	8
Soil chemical properties	
Total C (%)	6.2
Total N (%)	0.26
C:N ratio	23.9
pH	6.4
CEC (cmol/kg)	22
Exchangeable Na (cmol/kg)	0.53
Exchangeable K (cmol/kg)	1.68
Exchangeable Mg (cmol/kg)	2.77
Exchangeable Ca (cmol/kg)	12.6
Base saturation (%)	82
Olsen P (mg/kg)	69

Experimental design

The clonal tree material grown in the polyhouse was subjected to four water deficit treatments laid out in six blocks of five rows for one growing season (August 2009 to September 2010). Within each block of five rows, all four water deficit treatments were present plus one buffer row. Each treatment, which occupied one row, consisted of four trees. The treatments within each block were randomly assigned. The buffer row within each block was placed systematically on the next row to the north of rows subject to the summer drought treatment so that shading of the drought trees and thus any confounding influences were minimised. Trees were spaced 1.4 m apart so that there was no physical interaction between their crowns (Figure 2.1). Twenty four trees per treatment were present giving a total of 96 trees for the experiment.

Figure 2.1. View of the general layout of the polyhouse experiment.

Water deficit treatments

Four water deficit treatments were applied to the experimental trees. Irrigation was applied using drip sprinklers until pre-specified predawn needle water potentials were reached. The first treatment was 'well watered' with applications of water applied weekly between October and May (spring to autumn), and fortnightly between June and September (winter). The second treatment simulated 'extended summer drought' whereby water was withheld between November and April to allow drying of the soil profile. The drought was interrupted by the small, single application of water on March 1 to prevent tree mortality. Although predawn needle water potentials were not particularly low at this time, the trees had started showing large physical changes to continued drought. During the remainder of the year, water was applied at the same times and rates as the well watered treatment. The third and fourth treatments simulated 'early season cyclical drought' and 'late season cyclical drought', respectively. The early season cyclical drought treatment was applied between October and January (spring/early summer), while the late season cyclical drought treatment was applied between February and May (late summer/autumn). These treatments were timed to coincide with periods of expected early- and latewood development. Trees in these treatments were subjected to a series of drying cycles interrupted by water replenishment when needle water potential had fallen to approximately -0.5 MPa or lower. This water potential was

sufficient to generate a depressed growth response as previously observed in non-experimental trees. The early season drought trees were re-watered on November 7, December 1, December 26 and January 16, while the late season drought trees were re-watered on February 20, March 13 and April 19. When not subjected to the cyclical watering treatments between the stated months, water for both treatments was applied at the same times and rates as that for the well watered treatment. The length of time between watering for the cyclical drought treatments depended upon the time of year and air temperature. The time between watering varied from three to seven weeks during the October to May period.

Climatic variables

Climatic variables within the polyhouse were measured throughout the duration of the experiment. Variables measured were air temperature, relative humidity and photosynethically active irradiance (PAR). These variables were measured using sensors connected to a data logger (HOBO weather station, ONSET Computer Corporation, Bourne, MA, USA). Vapour pressure deficit (*D*) was calculated from measurements of air temperature and relative humidity (Allen *et al*., 1998). Soil temperature was measured monthly using a soil temperature probe 100 mm below the soil surface for all trees.

Air temperature fluctuated between -1 and 35 °C with a mean and standard deviation of 14.3 ± 5.9 °C. Monthly measures of soil temperature closely corresponded to mean air temperature throughout the course of the experiment (data not shown). Vapour pressure deficit fluctuated between 0.01 and 3.89 kPa with a mean and standard deviation of 0.41 ± 0.53 kPa, while the maximum value of photosynthetically active irradiance was 1023 μmol m^{-2} s^{-1} with a mean and standard deviation of 134 ± 234 μmol m^{-2} s^{-1} (Figure 2.2).

Measurements of tree growth

Measurements of tree growth were taken during the second year that the trees were planted. Monthly measurements of total tree height and diameter (at a tree height of 0.5 m) were made. Ground-line diameter was measured every second month. Both measures of diameter were made on a single axis for all trees using electronic calipers.

Sample needles were collected from current year needles from the youngest second order branches of 12 trees per treatment at the start and end of the experiment. These needles were bulked by block and nitrogen (N) concentration was measured using near-infrared spectroscopy

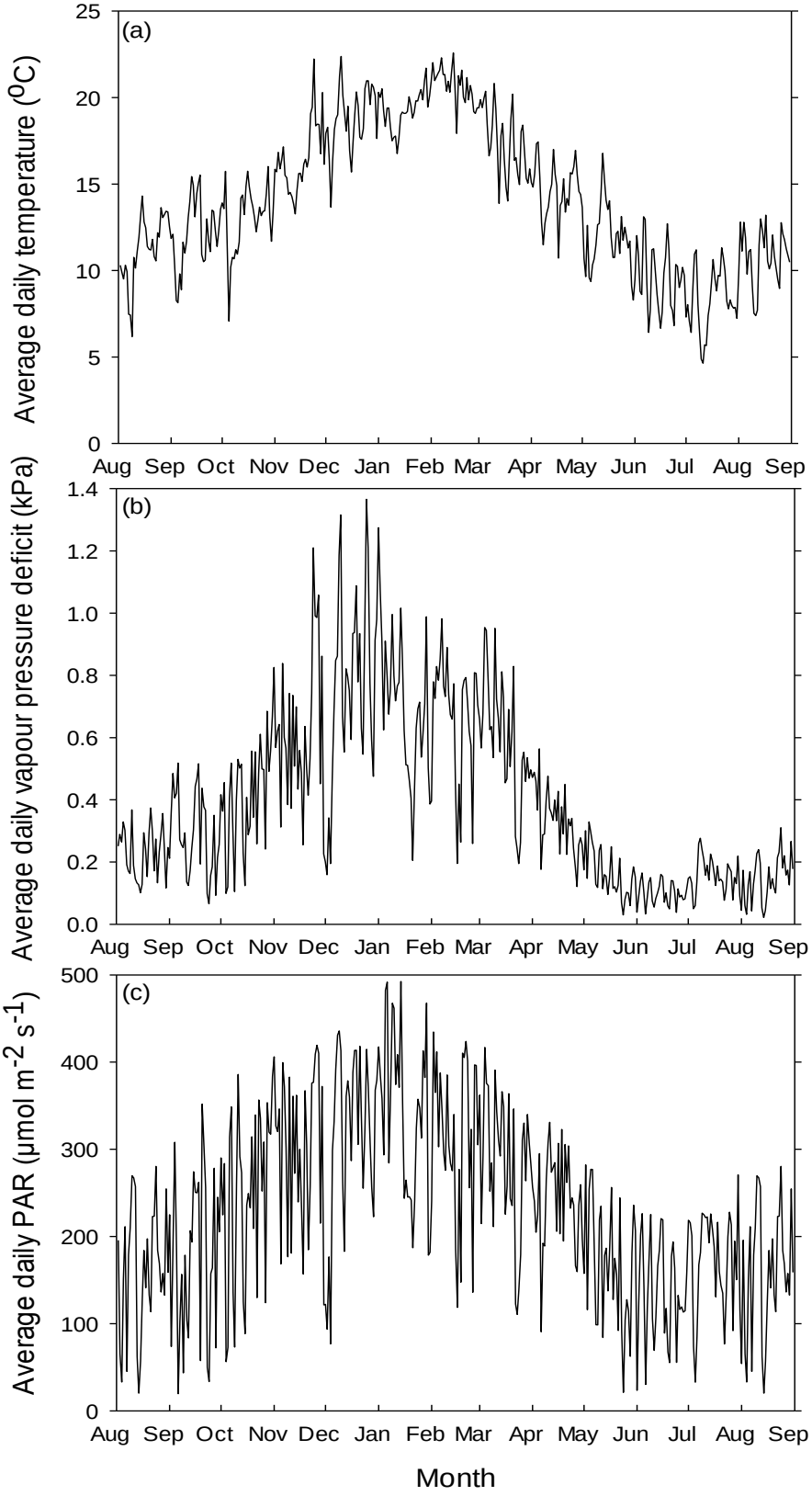


Figure 2.2. Climatic variation over the duration of the experiment showing annual variation in (a) average daily air temperature, (b) average daily vapour pressure deficit, and (c) average daily photosynthetically active irradiance (PAR).

calibrated by Dumas combustion, while phosphorus (P), potassium (K), sulphur (S), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and boron (B) were analysed using ICP-OES (Perkin Elmer Optima 3000DV, Waltham, MA, USA) after a HNO_3/H_2O_2 digestion. Whole tree litterfall from both current and second year needles was collected monthly, dried at 70 °C until constant weight was achieved and aggregated over the year for each treatment.

Predawn needle water potential

Measurements of predawn needle water potential (ψ_e) were made throughout the experiment using a pressure chamber. Three trees per treatment were sampled on each occasion. The timing of sampling depended on the season with weekly sampling occurring during spring, summer and autumn and fortnightly sampling occurring during winter. From each tree, three fascicles were collected from the youngest well developed foliage. The fascicles were collected before dawn and stored in test tubes on ice until ψ_e was measured, which was always within one hour of collection. Following Myers (1988), water stress integral (S_ψ) was determined as the cumulative integral of predawn needle water potential over the period which water deficits were imposed (1 October to 31 May). In addition to total values of S_ψ, monthly S_ψ was also calculated. Values of S_ψ are expressed as an absolute sum, so that larger values of S_ψ represent greater accumulated water deficits.

Physiological measurements

Photosynthetic rate (A), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured on fully expanded foliage of 12 trees in late autumn (May 30th, 2010) when the late season drought treatment was in effect. Measurements were made using the portable photosynthesis system, Li-6400 (Li-Cor, Lincoln, NE, USA). Transpiration was calculated from g_s and vapour pressure deficit (D). Three trees per treatment were measured. All measures were made during the course of one day. CO_2 concentration was maintained at 400 µmol mol^{-1} using the CO_2 mixer. Temperature, D and incident irradiance (Q) were left at ambient conditions during the period of testing with mean values and standard deviation of 20.8 ± 0.3 °C, 1.57 ± 0.04 kPa and 164 ± 10 µmol m^{-2} s^{-1}, respectively. For each tree, six needles were placed into the clear top cuvette ensuring no shading occurred between needles. Needles were left until values of A, g_s and C_i were stable and then values of A, g_s and C_i were recorded every 20 seconds for three minutes. All measures and analyses are presented on the basis of half total leaf surface area.

The response of photosynthesis (*A*), to incident irradiance (*Q*), was measured on fully expanded needles from 12 trees in late autumn (May 29th, 2010) using the Li-6400 system. Three trees per treatment were measured. For each tree, six needles were arranged in an enclosed cuvette avoiding shading between needles. Temperature in the cuvette was maintained at 18 °C, while the CO_2 concentration was maintained at 400 µmol mol^{-1} using the CO_2 mixer. The samples were equilibrated at an incident quantum flux (400-700 nm) of 2000 µmol m^{-2} s^{-1}, and measurements of photosynthesis were made as irradiance was reduced in 11 steps to 1500, 1100, 800, 600, 400, 300, 200, 150, 100, 50 and 0 µmol m^{-2} s^{-1}. At each level of *Q*, needles were left until values of *A*, g_s and C_i were stable and then *A* (µmol m^{-2} s^{-1}) was recorded before light intensity was dropped. All measurements and analyses are presented on the basis of half total leaf surface area.

The response of *A* to *Q* was fitted to the non-rectangular hyperbolic function (Farquhar and Wong, 1984) described by:

$$\theta(A + R_d)^2 - (\varepsilon Q + A_{max})(A + R_d) + \varepsilon Q A_{max} = 0 \quad (1)$$

where A_{max} is maximum rate of photosynthesis at saturating irradiance, R_d is rate of respiration in the dark, θ defines the convexity of the response curve, and ε, the initial slope of the curve, is the photosynthetic light-use efficiency.

Measurements of final tree growth, biomass and leaf area

At the conclusion of the experiment prior to destructive sampling, final measurements of tree height, diameter at 0.5 m up the stem and ground-line diameter were made for all trees. Stem slenderness was calculated as the ratio of tree height to ground-line diameter. Basal area was calculated using Husch *et al*. (2003). Crown dimensions were measured for all trees. The number of whorls, internode length, number of branches per whorl, branch diameter and branch length were measured. The largest branch diameter in each quadrant of the whorl was measured and branch diameter was defined as the mean of the largest branch in each of the four quadrants.

Total and component aboveground biomasses were determined by destructively harvesting 48 trees at the end of the experiment, representing 12 trees per treatment (two per block), and separated into stem, branch and foliage components. Aboveground biomasses were based on primary growth above the point of the apical meristem at the start of the second year of growth and implementation of the experimental treatments. The bulk root system was collected for 12 trees (three trees per treatment) in order to assess root structure. The 12 trees represented a range (low,

average and high) of aboveground biomass values for each treatment. All visible roots were recovered using a combination of sieving and floatation methods. The bulk root system was washed to minimise mineral soil contamination. Root growth was determined as the difference between initial and final root mass. Eight trees were destructively sampled at the start of the experiment to estimate initial root mass. All tree components were dried to 70 °C until constant mass was achieved, then weighed.

Leaf area was measured for 24 randomly selected trees (six per treatment, one per block) that were destructively sampled for dry matter partitioning. Leaf area was obtained by removing all foliage and separating this into age classes based on tree height at the start of the second year of growth and implementation of the experimental treatments. The year one leaf area incorporated the already existing untreated first season's growth, plus new growth from the second growing season, while year two leaf area consisted solely of the second season's new growth. Following Beets (1977), specific leaf area was determined from measurements of fascicle diameter, length and dry mass based on a subsample of 20 fascicles per age class. Values for leaf area are presented on the basis of half the total surface area. The number of fascicles per tree was estimated as total tree foliage dry weight divided by leaf area fascicle subsample dry weight. Samples for foliage nutrient concentrations were taken from the 48 trees that were not destructively sampled for biomass partitioning.

Foliar $\delta^{13}C$ isotope composition

Foliage stable carbon isotope ratios ($\delta^{13}C$) were measured for six trees per treatment (24 trees total) at the conclusion of the experiment. Oven dried foliage samples from the year two foliage of each sampled tree were ground. Using 3 mg of ground sample material, carbon isotope composition was determined using a Dumas Elemental Analyser (Europa Scientific ANCA-SL) interfaced to a Stable Isotope Mass Spectrometer (Europa Scientific Tracermass, Europa Scientific Ltd, Crewe, UK). The $^{13}C/^{12}C$ ratios are expressed as delta (δ) values in thousandths (‰). All analyses were run against the CSIRO sucrose standard ($\delta^{13}C$ = -10.8‰) and sample $\delta^{13}C$ was calculated relative to the Pee Dee Belemnite standard. The standard deviation of repeated $\delta^{13}C$ measurements of the laboratory samples was 0.3‰. The carbon isotope composition was calculated from:

$$\delta^{13}C\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000 \quad (2)$$

where R represents the ratio of $^{13}C/^{12}C$ of samples and standards, respectively (Brienen *et al*., 2011).

STATISTICAL ANALYSIS

All analyses were carried out using R software (R Core Team, 2013). There were no statistically significant treatment differences in any of the measured variables at the start of the experiment. The effect of water deficits on measured variables, except for estimates of physiological variables which were not assessed at the block level, were examined using a mixed effects model that included treatment as a fixed effect with block as a random effect as follows:

$$Y_{ij} = X_i \beta + Z_j b_j + e_{ij} \quad (3)$$

where Y_{ij} is the observation vector of the response variable, X is the fixed effect of treatment, Z is the random effect of block and e is the error term. The subscripts $_i$ and $_j$, represent treatment and block, respectively. β is the vector of fixed effect coefficients, and b is the vector of random effect coefficients. The random effect, b_j, and error, e_{ij}, are independent and normally distributed with mean zero and variances σ_b^2 and σ_e^2, respectively. Response variables included tree dimensions, crown and leaf characteristics, biomass partitioning and water use efficiency.

Linear models were developed for the early and late season drought treatments when examining the relationship between monthly water stress integral and monthly stem diameter growth using the following form:

$$y = a + bx \quad (4)$$

where y is monthly stem diameter growth, a is the intercept, b is the slope and x is monthly water stress integral. The slopes of the early and late season drought treatments were examined to see if they were significantly different at $P < 0.05$. A linear model was also developed to examine the relationship between woody biomass and foliage biomass, where foliage biomass was the response variable.

Tukey's HSD test was used, where applicable, to distinguish between treatment mean values. Differences were considered significant at $P < 0.05$.

RESULTS

Predawn needle water potential

The implementation of the early season cyclical drought, late season cyclical drought and summer drought treatments had a pronounced effect on ψ_e and induced consistently more negative values of ψ_e than for the well watered trees (Figure 2.3). The early season and late season cyclical drought treatments resulted in large fluctuations of ψ_e. In both cases, application of water improved ψ_e immediately and usually, dramatically. The lowest value of ψ_e achieved for the early and late season cyclical drought treatments occurred during the first drying-rewetting cycle. Compared with these treatments, the summer drought treatment induced consistently more negative values of ψ_e during the period between mid-December and April than the other treatments. Values of total S_ψ were 41.4, 66.8, 55.2 and 97.6 MPa-days for the well watered, early season drought, late season drought and summer drought treatments, respectively.

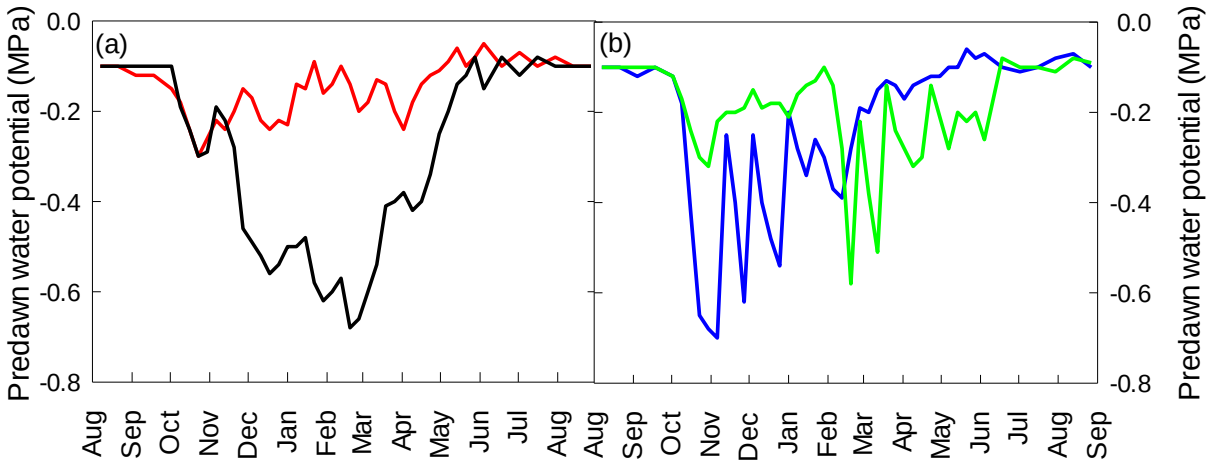


Figure 2.3. Seasonal changes in predawn needle water potential for (a) the well watered (red line) and summer drought (black line) treatments, and (b) early season cyclical drought (blue line) and late season cyclical drought (green line) treatments.

Tree characteristics

Tree height, both measures of diameter, and basal area were all significantly influenced by treatment ($P < 0.001$). Stem slenderness was also significantly influenced by treatment ($P = 0.012$), but only due to the variation in slenderness between the well watered and early season drought treatments. Basal area was the most sensitive indicator of water deficits (Table 2.2). With the

imposition of water deficits, marked divergence in monthly diameter and height growth became immediately apparent (Figure 2.4). Decreased growth for all water deficit treatments coincided with more negative predawn needle water potential values. Diameter growth for the summer drought treatment virtually ceased in March (0.04 mm growth for the month) whilst the well watered and early season drought treatments experienced their period of greatest incremental diameter growth (3.16 and 3.36 mm, respectively). After very different growth trajectories during the preceding months, rate of increment had converged for all treatments by mid winter (July). Height growth started increasing in July even though this month had the lowest daily temperatures. Rapid increases in height growth occurred the following month. Diameter growth started increasing in August. Compared to the well watered treatment, the summer drought treatment reduced height, diameter and basal area by 24.7%, 33.1% and 52.3%, respectively.

Table 2.2. Treatment variation in tree dimensions at the end of the experiment.

Treatment	Tree height (m)	Diameter (mm)	Ground-line diameter (mm)	Basal area (mm^2)	Slenderness ($m\ m^{-1}$)
Well watered	3.54 (0.55) a	38.26 (0.80) a	44.75 (0.96) a	1590 (76) a	79.6 (1.2) a
Early season	3.22 (0.53) b	32.37 (0.59) b	37.08 (0.60) b	1086 (35) b	87.0 (1.0) b
Late season	2.99 (0.59) b	30.61 (0.58) b	35.81 (0.64) b	1015 (38) b	83.8 (1.3) ab
Summer	2.61 (0.42) c	25.59 (0.42) c	31.02 (0.41) c	759 (21) c	84.1 (1.2) ab
Analysis of variance					
Treatment	37.3***	44.3***	45.3***	34.3***	5.12*

Each value is the treatment mean with standard error in parentheses for 24 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as F value followed by P category: *significant at $P < 0.05$; ***significant at $P < 0.001$.

Trees exposed to early season drought between October and January increased in diameter by 5.36 mm during that period compared with 10.60 mm for the late season drought trees and 10.17 mm for the well watered trees. When water deficits were imposed between February and May for the late season drought trees, their diameter increased by 2.37 mm compared with 8.90 mm for the early season drought and 9.41 mm for the well watered trees. The reduction in stem diameter growth was greater for the late season treatment than the early season treatment when compared with the well watered trees as illustrated in Figure 2.4(a). The early season drought trees recovered rapidly after water stress, and even surpassed the well watered trees for monthly stem height

growth. This suggests that late season drought was more detrimental to tree growth than early season drought.

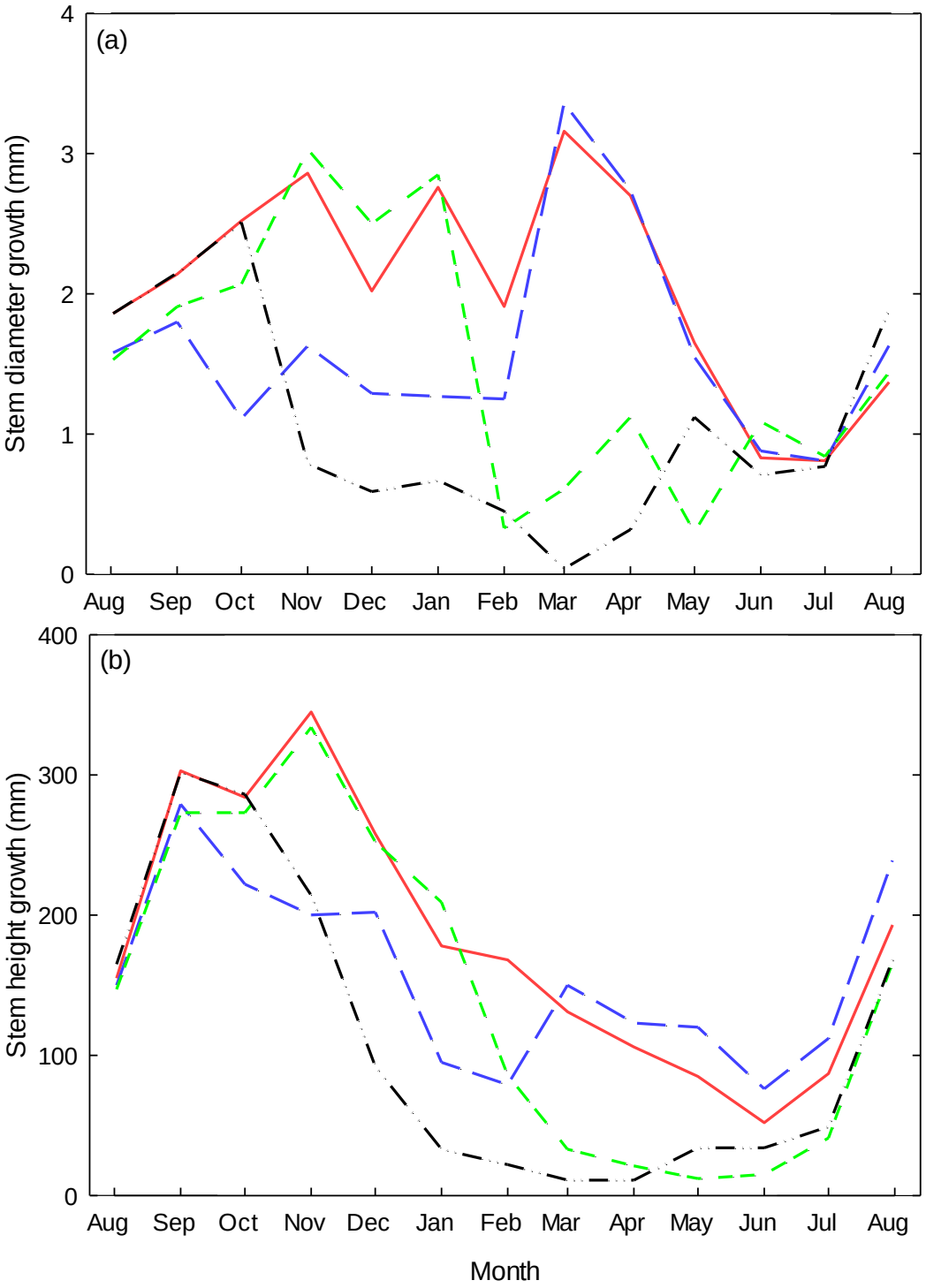


Figure 2.4. Average monthly incremental stem diameter growth (a) and height growth (b) for the well watered (solid red line), early season cyclical drought (long blue dash), late season cyclical drought (short green dash) and summer drought (black dash-dot-dot) treatments.

There were no significant differences (P> 0.05) in height, diameter and basal area between early and late season drought trees at the end of the experiment (Table 2.2) although total S_ψ was larger in the early season drought trees than in the late season drought trees. Monthly diameter growth was greater in the early season drought trees even though they were subjected to higher levels of water stress than those in the late season drought treatment. The relationship between monthly S_ψ and diameter growth is illustrated in Figure 2.5. Diameter growth was similar for both treatments irrespective of season during those periods when not subjected to water deficits and when monthly S_ψ values were comparable.

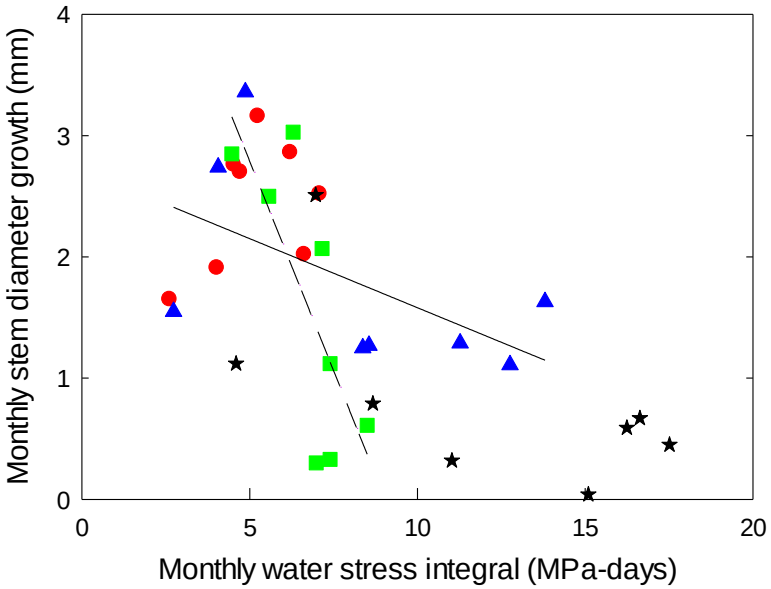


Figure 2.5. The relationship between monthly water stress integral (S_ψ) and monthly stem diameter growth for the period between October 2009 and May 2010 for the well watered (red circles), early season drought (blue triangle), late season drought (green square) and summer drought (black star) treatments. Best fit lines have been included for the early season drought (solid line, r^2 = 0.33) and late season drought (dashed line, r^2 = 0.57) treatments. The slopes were significantly different (P = 0.0365).

The primary effect of water deficits on crown characteristics were reductions in branch diameter and branch length (P < 0.001). The magnitude of these effects increased with the severity of the water deficit treatment with branch length varying by two-fold between the well watered and summer drought treatments (Table 2.3). The principal difference between the early and late season drought treatments was the number of fascicles per tree, otherwise crown variables were similar. Although summer drought produced a very similar number of whorls and branches per whorl as the other treatments, branch diameter and length were considerably different. This suggests that

summer drought does not significantly inhibit formation of lateral branching. Summer drought also had significantly shorter needles which were over 2.5 cm shorter than those of the well watered trees.

Table 2.3. Crown characteristics at the end of the experiment.

	Well watered	Early season	Late season	Summer	*Analysis of variance* Treatment
Number of whorls	2.29 (0.09) a	2.25 (0.11) a	2.25 (0.11) a	2.17 (0.10) a	0.50^{ns}
Internode length (cm)	61.77 (3.09) a	58.28 (3.10) ab	60.04 (2.21) ab	50.63 (2.96) b	3.28^{ns}
Branches per whorl	7.55 (0.21) ab	6.80 (0.18) a	7.59 (0.23) ab	7.73 (0.17) b	3.90*
Branch diameter (cm)	7.80 (0.16) a	6.28 (0.15) b	6.42 (0.16) b	4.93 (0.12) c	45.8***
Branch length (cm)	40.25 (1.93) a	29.19 (1.27) b	31.34 (1.68) b	20.76 (1.34) c	44.2***
Needle length (cm)	12.93 (0.35) a	12.10 (0.22) a	12.08 (0.46) a	10.34 (0.27) b	10.6***
Fascicles per tree	2643 (404) a	2297 (167) a	1704 (123) a	1547 (290) b	3.61*

Each value is the treatment mean with standard error in parentheses for 24 trees, except for needle length which is for 6 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as *F* value followed by *P* category: ^{ns}non-significant; *significant at $P < 0.05$; ***significant at $P < 0.001$.

Biomass partitioning

As expected, aboveground biomass component weights were influenced by treatment ($P < 0.001$), and gradients of productivity (tree size), however, stem, foliage and branch biomasses of the early season and late season drought treatments were not significantly different ($P > 0.05$) (Table 2.4). The difference in biomass between the well watered and summer drought treatments was most apparent for branch biomass which differed by a factor of just over 4, compared to 3.5 for stem and 2.6 for foliage. The relationship between woody biomass and foliage biomass was highly correlated ($r^2 = 0.91$) for all trees, irrespective of previous water status and tree size, indicating a strong functional relationship between conducting tissue of the tree and foliage (Figure 2.6).

The distribution of coarse (> 2 mm) and fine roots (< 2 mm) within the total root mass varied significantly ($P < 0.05$) with treatment (data not shown). The well watered and late season drought treatments had a high percentage of coarse roots (72% and 76%, respectively), whilst the early season and summer drought treatments had a lower fraction of coarse roots (57% and 59%,

respectively). For the latter treatments, the water deficits imposed earlier in the growing season were likely responsible for an allocation shift towards fine roots.

Table 2.4. Aboveground biomass for 48 destructively sampled trees by treatment.

	Tree component (g tree^{-1})			
Treatment	Stem	Branches	Foliage	Total
Well watered	350.08 (31.75) a	104.96 (12.87) a	291.39 (27.02) a	746.43 (67.82) a
Early season	212.82 (14.47) b	56.65 (4.99) b	206.46 (9.95) b	475.93 (27.07) b
Late season	187.08 (8.99) b	55.28 (4.75) b	169.97 (9.95) b	412.33 (20.46) b
Summer	100.15 (8.51) c	25.16 (3.32) c	111.64 (9.81) c	236.94 (19.94) c
Analysis of variance				
Treatment	36.3***	49.5***	52.9***	51.4***

Each value is the treatment mean with standard error in parentheses for 12 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as F value followed by P category: ***significant at $P < 0.001$.

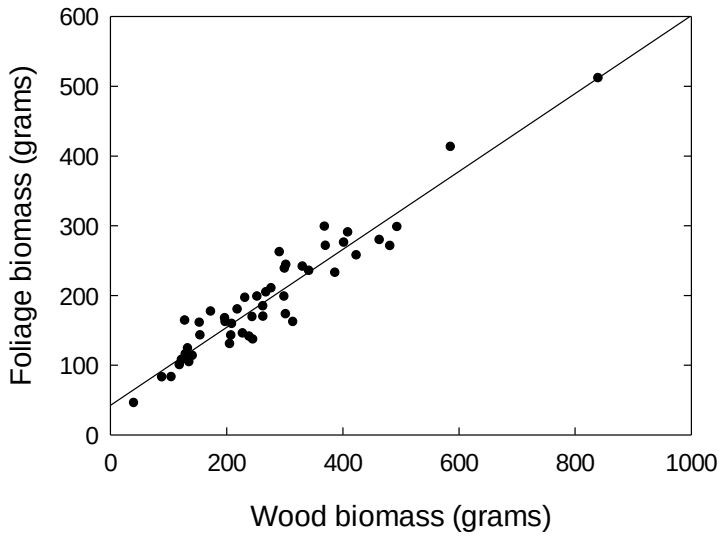


Figure 2.6. The relationship between wood biomass (stem and branch) and foliage biomass for 48 destructively sampled trees with line of best fit ($r^2 = 0.91$).

Ratios of aboveground component biomass to total biomass (Table 2.5) illustrated that by the end of the experiment the summer drought treatment had allocated comparatively more to foliage biomass and less to stem and branch biomass than other treatments. While no significant differences ($P > 0.05$) existed in allocation of biomass to stem between treatments, biomass allocated to branch and foliage components were significantly different ($P < 0.05$) between

treatments. Patterns of partitioning to biomass components followed water stress gradients with less water stressed trees allocating more to woody components and less to foliage than more water stressed trees.

Table 2.5. Variations in the ratio of stem, branches and foliage mass to total dry mass for 48 destructively sampled trees by treatment.

Treatment	Stem: total	Branches: total	Foliage: total
Well watered	0.473 (0.016) a	0.137 (0.009) a	0.389 (0.009) c
Early season	0.445 (0.012) a	0.117 (0.006) ab	0.437 (0.010) ab
Late season	0.455 (0.014) a	0.132 (0.005) a	0.412 (0.012) bc
Summer	0.425 (0.015) a	0.102 (0.007) b	0.472 (0.011) a
Analysis of variance			
Treatment	2.57^{ns}	7.98**	12.91***

Each value is the treatment mean with standard error in parentheses for 12 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as *F* value followed by *P* category: ^{ns}non-significant; **significant at $P < 0.01$; ***significant at $P < 0.001$.

Leaf characteristics

Leaf area varied considerably among treatments. A significant treatment effect was observed for year one leaf ($P < 0.001$), year two leaf ($P < 0.01$) and total leaf area ($P < 0.0001$). Although water deficits were only imposed during the second year, the effect on both the growth and development of the year two foliage and the continued growth and retention of the year one foliage was pronounced (Figure 2.7). Year two foliage leaf area for the well watered treatment was double that of the summer drought treatment. Leaf areas for one-year-old, two-year-old and total foliage were not significantly different between the early season and late season drought treatments ($P > 0.05$). Values of total leaf area of the early season, late season and summer drought treatments were 71%, 73% and 60%, respectively, of that for the well watered treatment.

Needle litterfall varied monthly (summarised to quarterly values) and was significantly ($P < 0.01$) affected by treatment (Table 2.6). The impact of cyclical drought on the early season treatment ceased early enough to prevent needle loss, however, the late season treatment showed significant needle loss towards the end of the treatment period. The foliar nutrient status of the trees does not suggest any direct nutritional problems with the more water stressed trees, although differences in

the nitrogen/phosphorus (N/P) ratio between the well watered (3.78) and summer drought (6.12) treatments were present (Table 2.7).

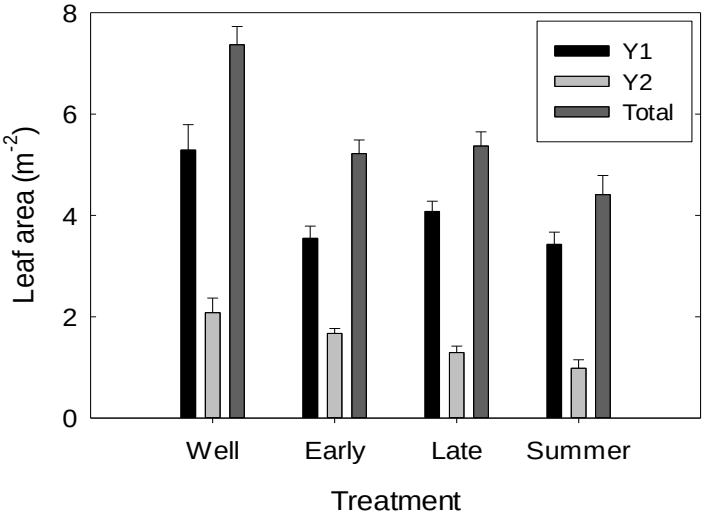


Figure 2.7. Leaf area for year one, year two and total tree foliage for the well watered, early season drought, late season drought and summer drought treatments.

Table 2.6. Quarterly needle litterfall (g tree^{-1}) for whole tree foliage.

Treatment	Aug - Oct	Nov - Jan	Feb - April	May - July	Total litterfall	Litterfall as % of foliage biomass
Well watered	0.29 (0.05) a	0.36 (0.07) ab	1.57 (0.30) ab	2.82 (0.43) b	5.03 (0.65) ab	1.73 (0.28) bc
Early season	0.30 (0.06) a	0.27 (0.08) ab	0.62 (0.15) b	0.72 (0.12) c	1.90 (0.29) b	0.92 (0.14) c
Late season	0.19 (0.05) a	0.18 (0.04) b	1.32 (0.34) b	5.18 (0.84) a	6.88 (1.14) a	4.05 (0.59) b
Summer	0.24 (0.05) a	0.46 (0.10) a	3.18 (0.67) a	3.21 (0.61) b	7.09 (1.26) a	6.35 (1.16) a
Analysis of variance						
Treatment	0.87^{ns}	3.39*	6.26**	14.8***	9.31**	17.5***

Each value is the treatment mean with standard error in parentheses for 12 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as F value followed by P category: ^{ns}non-significant; *significant at $P < 0.05$; **significant at $P < 0.01$; ***significant at $P < 0.001$.

Table 2.7. Foliar nutrient status at beginning and by treatment at end of the experiment.

Variable	Start of experiment	Well watered	Early season	Late season	Summer drought
Nitrogen (%)	1.80	1.40	1.67	1.53	1.53
Phosphorus (%)	0.14	0.37	0.35	0.29	0.25
Potassium (%)	1.60	1.20	1.23	1.10	0.97
Sulphur (%)	0.24	0.24	0.22	0.23	0.22
Calcium (%)	0.38	0.23	0.21	0.20	0.28
Magnesium (%)	0.11	0.10	0.09	0.09	0.10
Sodium (%)	0.01	0.01	0.01	0.01	0.01
N:P	12.86	3.78	4.77	5.28	6.12
N:K	1.13	1.17	1.36	1.39	1.58
K:Mg	14.55	12.00	13.67	12.22	9.70
Iron (mg/kg)	72	86	82	95	111
Manganese (mg/kg)	51	174	147	128	127
Zinc (mg/kg)	63	65	59	57	58
Copper (mg/kg)	4	5	6	5	5
Boron (mg/kg)	25	20	21	16	15

Each value is the treatment mean for three bulk samples. Each bulk sample represented foliage from two blocks of the same treatment.

Physiological measurements

Response of photosynthesis (*A*) to incident irradiance (*Q*) varied considerably depending on tree water status. Values of *A* across the range of *Q* were lower in the late season drought treatment which at time of measurement in May 2010, had gone six weeks without water replenishment compared to one week for the remaining treatments. For the well watered, early season drought and summer drought treatments, *A* increased rapidly with increasing *Q* up to 500 µmol m^{-2} s^{-1}, then continued to increase without reaching full saturation even when *Q* reached 2000 µmol m^{-2} s^{-1} (Figure 2.8). The A_{max} for these treatments was 13.30, 14.64 and 13.66 µmol m^{-2} s^{-1}, respectively (Table 2.8). In contrast, the late season drought treatment showed a slight increased in *A* in response to increasing *Q*, reaching a A_{max} of 3.65 µmol m^{-2} s^{-1}. Values of photosynthetic light-use efficiency, ε, were in the range of 51 to 65 mmol CO_2 mol^{-1} for the well watered, early season drought and summer drought treatments. For the late season drought treatment, ε was considerably lower at 16.2 mmol CO_2 mol^{-1}.

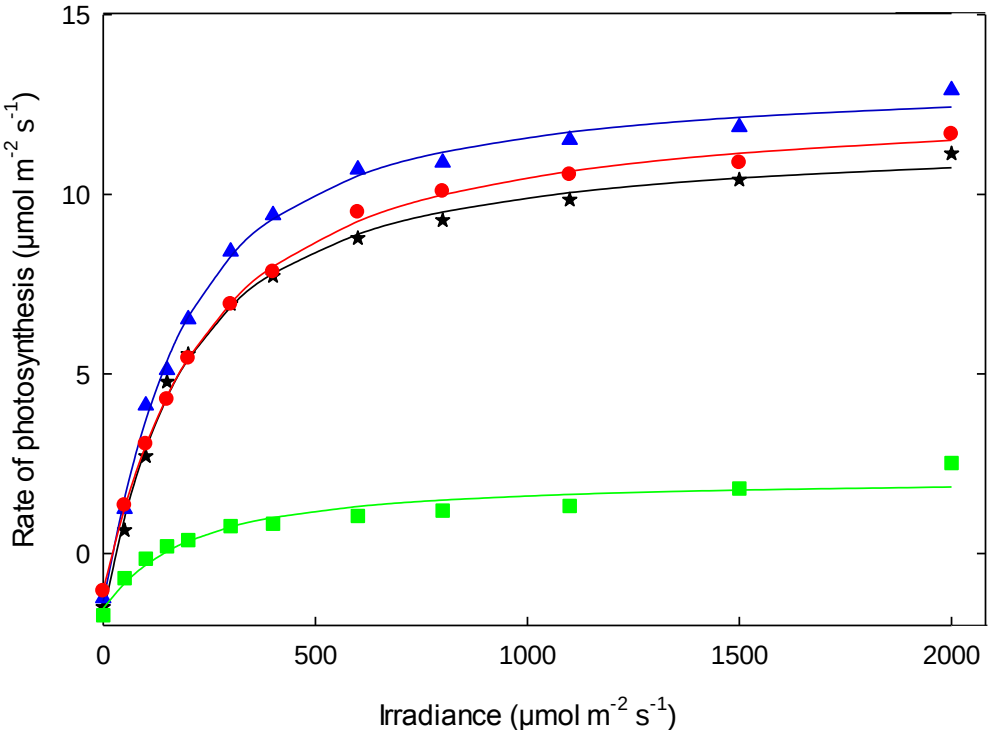


Figure 2.8. Relationship between photosynthesis and irradiance for the well watered (red line), early season cyclical drought (blue line), late season cyclical drought (green line) and summer drought (black line) treatments. The curves are generated from the non-rectangular hyperbola given in Equation 1 based on the mean parameters for each treatment.

Table 2.8. Parameter values for the fitted response of *A* to *Q*.

Treatment	A_{max} (µmol m^{-2} s^{-1})	R_d (µmol m^{-2} s^{-1})	θ	ε (mmol CO_2 mol^{-1})
Well watered	13.30	1.65	0.38	61.4
Early season	14.64	1.33	0.46	64.6
Late season	3.65	1.53	0.30	16.2
Summer	13.66	1.00	0.34	51.2

Each value is the fitted value based on three trees per treatment for maximum rate of photosynthesis (A_{max}), rate of respiration in the dark (R_d), the convexity of the response curve (θ), and photosynthetic light-use efficiency (ε).

Measurements of *A*, g_s, C_i and transpiration for the late season drought treatment were all substantially hindered at time of measurement in late autumn (Table 2.9), none more so than for g_s. Significant treatment differences existed for measures of g_s, C_i and transpiration ($P < 0.05$). The

greater change in values for g_s compared with A resulted in a considerably lower A/g_s ratio for the late season drought treatment than the other treatments. This retardation demonstrated the impact of water deficits on physiological processes and corresponded to reduced diameter and height growth at this time for the late season drought treatment compared with the other treatments.

Table 2.9. Estimates of physiological variables by treatment.

Treatment	A (µmol m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})	C_i (µmol m^{-2} s^{-1})	Transpiration (µmol m^{-2} s^{-1})
Well watered	7.54 (0.78) a	116.75 (6.29) a	281.52 (6.79) a	1.43 (0.25) ab
Early season	7.18 (1.17) a	134.20 (23.90) a	298.16 (5.43) a	1.72 (0.40) a
Late season	4.38 (0.17) a	36.43 (12.10) b	113.24 (35.87) b	0.50 (0.11) b
Summer	6.83 (0.58) a	100.26 (6.66) a	275.10 (7.52) a	1.14 (0.02) ab
Analysis of variance				
Treatment	3.50^{ns}	10.3**	53.2***	4.77**

Each value is the treatment mean with standard error in parentheses for three trees for photosynthetic rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as F value followed by P category: ^{ns}non-significant; **significant at $P < 0.01$; ***significant at $P < 0.001$.

Estimates of water use efficiency

Significant treatment differences in $\delta^{13}C$ ($P = 0.0375$) between the well watered and summer drought treatments were observed. The well watered trees showed significantly lower $\delta^{13}C$ values over the entire growth period compared to those for the summer drought trees because of higher soil water availability (Figure 2.9). The summer drought treatment had the most enriched values of $\delta^{13}C$, with an average of -25.93‰ compared to -26.57‰, -26.65‰ and -27.20‰ for the late season, early season and well watered treatments, respectively. The magnitude of variation within the early season drought treatment (3.06‰) was much greater than the variability among treatments (1.27‰). Across all samples, foliar $\delta^{13}C$ ranged from -25.29‰ to -28.63‰ (range of 3.14‰).

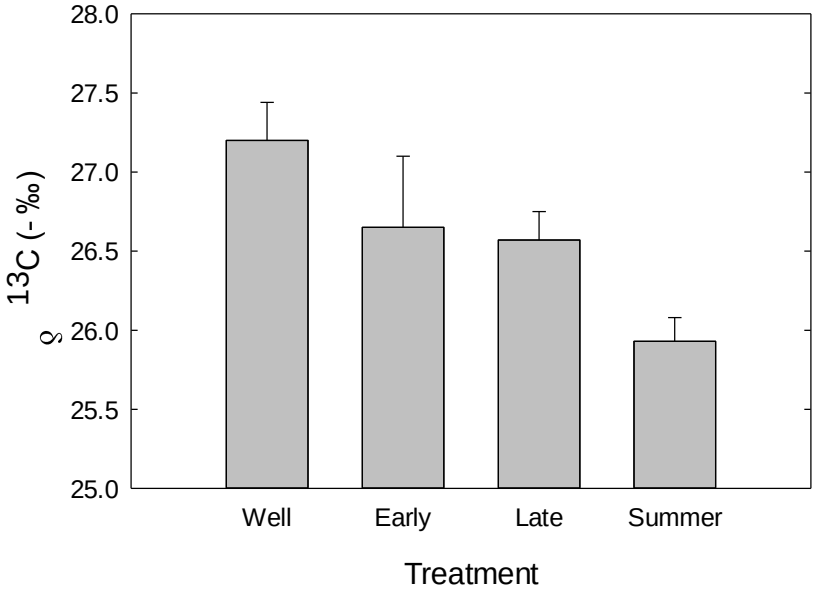


Figure 2.9. Average $\delta^{13}C$ of year two foliage by treatment, with standard error bars.

DISCUSSION

Previous experiments on dry sites that included irrigation have shown that water deficits limit tree growth by regulating the period during which tree growth is possible (Benson *et al*., 1992). Growth of trees in the well watered treatment was largely unaffected by water stress as confirmed from measurements of predawn needle water potential, which did not fall below -0.30 MPa. Although differences between treatments in predawn needle water potentials were often quite small, Myers (1988) showed that small differences maintained over long periods can lead to large changes in growth. Improved water availability led to increased growth and distinct differences were observed between the well watered, cyclical drought and summer drought treatments. Findings in this study generally agree with those of other studies showing the importance and positive effect of increased water availability on *P. radiata* growth (Linder *et al*., 1987; Benson *et al*., 1992; Snowdon and Benson, 1992; Watt *et al*., 2003; Waterworth *et al*., 2007; Alvarez *et al*., 2013).

The higher slope for the late season drought treatment in Figure 2.5 means that similar monthly water stress integral had a more pronounced effect on monthly stem diameter growth compared with the early season drought treatment, whilst demonstrating that the early season drought trees experienced greater levels of water stress (total S_{ψ} = 66.8 vs. 55.2 MPa-days). In addition, the early season drought trees rapidly recovered from water stress and exceeded the well watered treatment for monthly stem height growth, whilst the late season drought trees remained suppressed through to the end of the experiment and into the new growing season. These results

demonstrate the complex interactions between the phenology of foliage production, partitioning of photosynthates and the availability of resources during different seasons. Although the early and late season drought treatments were implemented at different times of the year when different factors may have been influencing tree growth such as air temperature and vapour pressure deficit, comparisons with the well watered trees as the control treatment allow for direct comparisons between the relative effects of early and late season drought on physical measures of tree growth.

Furthermore, late season drought would undoubtedly impact on tree growth during the following season compounding the issue of reduced tree growth. Cambial activity and xylem cell development are considerable sinks of energy and particularly demanding in sucrose from photosynthesis (Hansen and Beck, 1994; Oribe *et al*., 2003), so the effects of drought on photoassimilates can manifest in reduced wood production (Arend and Fromm, 2007). Previous studies have shown that early season wood formation depends strongly on previously stored carbohydrates (Kagawa *et al*., 2006) and drought towards the end of the growing season affects the formation of needle initials, hence affecting the potential production of needle area in the following year (Linder, 1987). Thus, the first flush and early season wood is expected to reflect growing conditions in the previous year more than conditions early in the current year (Porte and Loustau, 2001). This may be more applicable in *P. radiata* for juvenile trees which have a greater tendency to be monocyclic than mature trees which tend to be polycyclic as they mature (Mead, 2013).

Diameter growth rates of the well watered trees remained high until late autumn, however, there was a decline in incremental diameter growth for the summer months of December and February which was possibly caused by atmospheric conditions. The highest values of vapour pressure deficit occurred in early summer (December) and the highest daily average temperatures in late summer (February). When the vapour pressure deficit increases, stomatal conductance decreases (Beneke, 1980), thus reducing photosynthesis and it has been shown that cell diameters decrease in the radial direction in *P. radiata* when the temperature is above 15 °C (Jenkins, 1975). These reductions in rate of diameter growth, more so for December than February, coincide with the main period of needle elongation. The foliage would thus have been a strong competing sink for assimilates, reducing the resources available for diameter growth (Linder *et al*., 1987). Diameter growth rates for the summer drought treatment virtually ceased during March, with many of the individual trees within that treatment actually experiencing stem shrinkage. During this period, predawn needle water potential for this treatment was approximately -0.7 MPa, demonstrating that even at needle water potentials that are not particularly low, stem growth can cease and stem shrinkage can occur.

Distinct differences in biomass were observed between the well watered, cyclical drought and summer drought treatments. The trend of increasing component and total biomass with improved water availability is similarly seen in numerous other conifer studies (Ledig *et al*., 1970; Axelsson and Axelsson, 1986; Gower *et al*., 1992; Snowdon and Benson, 1992; Woods *et al*., 1992; Yunusa *et al*., 1995*b*; Albaugh *et al*., 1998; Watt *et al*., 2003; Espinoza *et al*., 2013). The well watered trees in this study had over three-fold more total biomass than the summer drought trees and a four-fold difference in branch biomass which exceeded the difference for any other tree components between these treatments. This was driven by differences in branch diameter and length and reflects the decreased ratio of branches to total biomass observed in the summer drought treatment. A clear trend of increased partitioning of biomass to foliage over branch and stem components with increasing levels of water stress was observed in this experiment in contradiction to general allocation theory which states that partitioning should be in favour of organs involved in water uptake and transport (McConnaughay and Coleman, 1999; Litton *et al*., 2007).

The strong positive relationship between woody biomass and foliage biomass conforms to the pipe model theory advocated by Shinozaki *et al*. (1964*a*, 1964*b*). They concluded that a given unit of foliage was supported by a continuation of conducting tissue of constant cross-sectional area, analogous to a pipe system. This observation has been interpreted in terms of a hydraulic theory of tree functioning, although it has been found to be only approximately true (Berninger *et al*., 2005). Across all treatments and tree sizes in this experiment, this relationship held and suggests that in juvenile *P. radiata*, estimates of canopy leaf area might be made from the sapwood area at the base of the tree. However, results should be treated with caution as the polyhouse grown trees may not be representative of field grown trees.

Water deficits resulted in smaller needles, reduced leaf area and considerably smaller crowns. Smaller crowns were the result of reduced branch length and diameter and a reduced number of fascicles. Pronounced differences existed for year one, year two and total leaf area across all treatments. Water deficits impacted on the growth and retention of year one foliage as well as the formation and growth of the year two foliage. The greater second season (year two) leaf area for the early season drought trees compared to the late season drought trees was not expected, even taking into account increased needle litterfall for the late season drought trees, as early season drought was expected to retard continued formation and growth of needles. However, it has been shown that needles on later season flushes can continue to develop until all aboveground growth sinks are halted and at the same rate as those earlier in the growing season (Rook *et al*., 1987; Dougherty *et al*., 1994).

A positive effect of water in irrigation experiments can be due to increased nitrogen mineralisation and transport of nitrogen to roots, rather than a physiological effect of water on the trees (Högberg *et al*., 1993). The nutrient status of the foliage was reasonably similar for all treatments and does not suggest any direct nutritional problems with the more water stressed trees, although differences were seen in the N/P ratio between the well watered (3.78) and summer drought (6.12) treatments. However, interpretations of nutrient uptake based on foliar nutrient concentrations must be made with great caution. High growth rates may result in dilution of nutrients, or alternatively, growth limitations imposed by constraining environmental or nutritional factors may result in elevated foliar concentrations of the nutrient of interest. The colour of the foliage varied noticeably between the dark green well watered trees and the yellow-green summer drought trees by the end of the experiment. Plants can appear chlorotic (yellow-green) when suffering moderate or severe drought. This is caused by phyto-oxidative damage to photosynthetic apparatus and chlorophyll due to excess energy producing highly reactive molecules, and has been noted previously in plant water stress studies (Nir and Poljakoff-Mayber, 1967).

Imposed water deficits at time of measurement had a pronounced effect on A_{max}, ε and the rate of increase of *A* for late season drought trees. It is well established that soil moisture deficits severely restrict gas exchange (Benecke, 1980) and although measurements of *A* were only made in late autumn, they clearly demonstrated the effect of water stress on physiological measures. Measures of *A*, g_s, C_i and transpiration were all significantly reduced for the late season drought treatment at time of observation. The considerably lower A/g_s ratio for the late season drought treatment suggests that water deficits resulted in greater changes in stomatal conductance than in photosynthesis. While measures were only made in late autumn, the benefit of testing at one point in time is the reduction in large fluctuations in temperature and vapour pressure deficit that arise from testing at different times of the year and the subsequent impact of these environmental variables on *A* and g_s.

$\delta^{13}C$ in plant foliage is determined by both stomatal conductance and photosynthetic capacity (Farquhar *et al*., 1989) and thus, $\delta^{13}C$ is an indicator of stomatal activity throughout the period the leaf tissue was synthesised (McNulty and Swank, 1995; Barbour *et al*., 2002; Dawson *et al*., 2002). In response to water deficits, stomatal conductance decreases to prevent excessive water losses, leading to reduced values for C_i and lower ^{13}C discrimination (Farquhar and Sharkey, 1982). Since stomatal conductance is proportional to water loss (given similar ambient conditions), $\delta^{13}C$ is positively correlated with water use efficiency (Farquhar and Richards, 1984). Thus, given that the summer drought treatment had the most enriched values of $\delta^{13}C$, the results imply that the summer drought treatment had the greatest water use efficiency and as such, fixed the most carbon per unit

amount of water transpired. Under water stress, diameter and height growth, aboveground biomass and foliage area were significantly reduced, while water use efficiency was significantly increased. Numerous studies have suggested that $\delta^{13}C$ is more strongly influenced by stomatal conductance than photosynthetic capacity in *P. radiata* (Korol *et al*., 1999; Rowell *et al*., 2009), particularly when soil water content is low (Walcroft *et al*., 1997). Given that stomatal conductance appears to have exhibited greater change than photosynthetic capacity in this study, it is likely that $\delta^{13}C$ was influenced in a similar manner as observed by Korol *et al*. (1999) and Rowell *et al*. (2009).

CONCLUSIONS

Strong positive interactions between water availability and growth of juvenile *P. radiata* were observed in this experiment. Summer drought reduced height, diameter and basal area by 24.7%, 33.1%, and 52.3%, respectively, while aboveground biomass was reduced by 68.3% and total leaf area by 40.0%, compared with the well watered treatment. Treatment effects were almost always significant for all variables tested ($P < 0.05$) but not between the early and late season drought treatments. Branch length was the most affected crown characteristic, reducing by 10 cm for the early and late season drought treatments, and by 20 cm for the summer drought treatment, compared with the well watered treatment. The summer drought treatment had the most enriched values of $\delta^{13}C$, with an average of -25.93‰, indicating greater water use efficiency, compared to -26.57‰, -26.65‰ and -27.20‰ for the late season, early season and well watered treatments, respectively. At similar monthly water stress integral, late season drought trees experienced a greater reduction in monthly stem diameter growth compared to the early season drought trees, suggesting that late season drought is more detrimental to tree growth and biomass accumulation in juvenile *P. radiata* than early season drought. The results herein clearly demonstrate the benefit to forest managers of improved water availability to juvenile *P. radiata* to ensure sound establishment and vigorous growth and can be useful for improving both silvicultural and environmental approaches to forest management.

CHAPTER THREE

CARBON FLUX AND PARTITIONING IN JUVENILE *PINUS RADIATA* IN RESPONSE TO WATER DEFICITS

SUMMARY

Quantifying carbon partitioning between above- and belowground components is essential for understanding mechanisms driving the carbon balance of *Pinus radiata* D. Don plantations. Carbon (C) flux and partitioning responses of *Pinus radiata* to the timing and duration of water deficits were estimated in two-year-old trees growing in a controlled environment over the course of one growing season. We used a carbon balance approach to quantify growth, carbon flux and carbon partitioning in response to well watered, early season cyclical drought, late season cyclical drought and summer drought treatments. We examined how the timing and duration of water deficits modified gross primary production (GPP), net primary production (NPP), carbon fluxes to aboveground net primary production (ANPP) and total belowground carbon flux (TBCF), and the partitioning of GPP to ANPP and TBCF. Water deficits significantly decreased fluxes to GPP, NPP, ANPP and TBCF following gradients of tree productivity. Although the early season drought treatment experienced greater levels of water stress, growth and productivity were superior to the late season drought treatment. Treatment values of GPP were between 1470 and 4142 g C per tree. Partitioning of GPP to ANPP and TBCF was not affected by treatment and partitioning of GPP was predominantly to TBCF (45 - 56%) for all treatments. Partitioning of TBCF to soil respiration (F_S) did not significantly differ with treatment but F_S was the dominant component of TBCF (77 - 88%) and the carbon budget for all treatments. These results provide insight into how the timing and duration of water deficits regulate carbon fluxes and partitioning in individual trees and suggest that water deficits are more important in delaying growth than in changing patterns of carbon allocation in *P. radiata*.

INTRODUCTION

Carbon partitioning plays a critical role in forest ecosystem carbon cycling by shifting the products of photosynthesis between respiration and biomass production (Litton *et al*., 2007). Predicting how forest growth and ecosystem carbon storage respond to changes in resource supplies remains a challenge, particularly because the environmental regulation of the components of the carbon balance are poorly understood. This lack of understanding of carbon partitioning limits our ability to model the forest carbon cycle, predict the effects of global climate change on carbon cycling and, most importantly for forest managers of *Pinus radiata* D. Don plantations, to accurately predict forest productivity for new climates, sites and genotypes (Ryan *et al*., 2010). If global climate change produces more frequent occurrences of drought, then the response of forests to water deficits and the potential consequences of shifting carbon fluxes on carbon storage will become important considerations for forest managers.

The general hypothesis underpinning existing carbon allocation theory is that plants maximise growth by partitioning carbon to various plant organs to optimise the capture of limiting resources (McConnaughay and Coleman, 1999; Litton *et al*., 2007). In response to water deficits, many species exhibit changes in partitioning in favour of the organs involved in water uptake and transport, and increases in water use efficiency (Pereira and Chaves, 1993). For instance, an increase in the ratio of root biomass to leaf biomass would be expected. As a result, increased fluxes to belowground organs at the expense of aboveground organs is often observed (Bongarten and Teskey, 1987; Comeau and Kimmins, 1989; Johnson, 1990; Gower *et al*., 1992; Albaugh *et al*., 1998; Coyle and Coleman, 2005), however, this is not always the case (McConnaughay and Coleman, 1999; Ryan *et al*., 2010).

Previous studies of *Pinus radiata*, a species relatively sensitive to water deficits (Benecke, 1980; Sands and Nambiar, 1984; Watt *et al*., 2003), have shown that decreased availability of water reduces above- and belowground net primary production (Snowdon and Benson, 1992; Woods *et al*., 1992; Yunusa *et al*., 1995*b*; Watt *et al*., 2003; Espinoza *et al*., 2013). However, partitioning of carbon to aboveground components is often contradictory between studies with Snowdon and Benson (1992) observing increased partitioning to stem wood production, whilst Watt *et al*. (2003) noted increased partitioning to branch wood production, in response to increased water availability. Information on belowground carbon flux and partitioning in *P. radiata* in response to water availability is very sparse.

Studies typically only examine above- and belowground distribution of biomass (e.g. Bongarten and Teskey, 1987; Steinberg *et al*., 1990; Ingestad and Agren, 1991; Albaugh *et al*., 1998;

McConnaughay and Coleman, 1999; Chambel *et al*., 2007), yet the standing crop represents a fraction of total carbon expended, and thus our understanding of carbon partitioning in forest ecosystems is limited (Litton *et al*., 2007). Much of the carbon that is assimilated by trees is respired, allocated to mycorrhizae, exuded by roots or released by above- and belowground litter, and these processes are poorly quantified and controls on them are poorly understood (Ryan, 1991*a*; Giardina *et al*., 2003). The allocation of carbon belowground has received little attention (Newman *et al*., 2006; Marsden *et al*., 2008) yet carbon loss by respiration from soil is the second largest carbon flux in terrestrial ecosystems, second only to gross primary production (Raich and Schlesinger, 1992). Typically, soil respiration shows large spatial and temporal variability, both within and among sites (Raich and Nadelhoffer, 1989; Hanson *et al*., 1993) and is primarily influenced by soil temperature and soil water availability (Carlyle and Than, 1988; Raich and Schlesinger, 1992; Howard and Howard, 1993; Raich and Potter, 1995; Davidson *et al*., 1998).

Carbon fluxes and partitioning in forest ecosystems have been studied using methodologies proposed by Raich and Nadelhoffer (1989) and Ryan (1991*b*). More recent improvements for estimating carbon flux to roots (Haynes and Gower, 1995; Giardina and Ryan, 2002) have assisted with these methodologies. Using these methods, gross primary production (GPP) is partitioned into its measured or estimated component fluxes. GPP, which is the annual sum of carbon allocated to dry matter production and respiration can be partitioned into aboveground net primary production (ANPP: sum of foliage, branch, bark and stem production), aboveground plant respiration (APR) and total belowground carbon flux (TBCF: sum of carbon fluxes corresponding to the production and respiration of coarse and fine roots, exudates and root turnover, and carbon pool changes).

Quantifying carbon partitioning between above- and belowground components is essential for understanding mechanisms driving the carbon balance of juvenile *P. radiata*. Better knowledge of fluxes and partitioning to woody and non-woody tissues in response to water deficits would greatly enhance our ability to predict patterns of productivity and could provide opportunities to increase forest productivity. The primary objective of this experiment was to examine the effect of seasonal water deficits on carbon assimilation and partitioning to above- and belowground components in juvenile *P. radiata* using a carbon balance approach. We examined the impact of the timing and duration of water deficits on absolute and relative values of GPP, net primary production (NPP), ANPP, APR and TBCF. Specifically for testing functional balance hypothesis, we used the components of the carbon balance that we actually measured (i.e. ANPP, ΔC_R, F_S) rather than using the variables for which we made an assumption (i.e. APR). Therefore, our functional balance hypotheses were that (i) carbon partitioning belowground would increase with water stress, i.e. the TBCF/ANPP ratio would increase with water stress, (ii) roots of water stressed trees would

proportionally respire more than roots of non-stressed trees, i.e. F_S / ΔC_R would increase with water stress, and (iii) carbon partitioning belowground would be similar for the early and late season drought treatments.

METHODS

Experimental design and treatments

The experiment was undertaken in a polyhouse which provided growing conditions where water supply to *Pinus radiata* D. Don clonal stock could be controlled. The clone used was a high wood density, high acoustic velocity clone (Clone 35, Forest Genetics CellFor Ltd) produced using somatic embryogenesis. The nursery raised trees were planted in the polyhouse for a two year period and the experimental treatments were applied during the second year. The trees were planted in a free draining loamy sand soil in 200 litre pots to reduce any potential for water logging and root binding. Soil texture and particle size were determined prior to experiment commencement using a hydrometer for smaller particles and sieve for larger particles. Particles were separated into <0.002, 0.002-0.06, 0.06-0.2, 0.2-0.6 and 0.6-2.0 mm size classes. Medium and coarse sand compromised 76% of the soil.

The clonal tree material grown in the polyhouse was subjected to four water deficit treatments laid out in six blocks of five rows for one growing season (August 2009 to September 2010). Within each block of five rows, all four water deficit treatments were present plus one buffer row. Each treatment, which occupied one row, consisted of four trees. The treatments within each block were assigned randomly. The buffer row within each block was placed systematically on the next row to the north of rows subject to the summer drought treatment so that shading of the drought trees and thus any confounding influences were minimised. Trees were spaced 1.4 m apart so that there was no physical interaction between their crowns. Twenty-four trees per treatment were present giving a total of 96 trees. For this experiment, measurements were made on the middle two trees within each row. In total, 48 trees comprising 12 trees per treatment were assessed. As a result, tree dimensions of these 12 trees differ from those of the 24 trees per treatment assessed in Chapter 2.

Four water deficit treatments were applied to the experimental trees. Irrigation was applied using drip sprinklers until pre-specified predawn needle water potentials were reached. The first treatment was ‘well watered’ with applications of water applied weekly between October and May (spring to autumn), and fortnightly between June and September (winter). The second treatment

simulated 'extended summer drought' whereby water was withheld between November and April to allow drying of the soil profile. The drought was interrupted by the small, single application of water on March 1 to prevent tree mortality. Although predawn needle water potentials were not particularly low at this time, the trees had started showing large physical changes to continued drought. During the remainder of the year, water was applied at the same times and rates as the well watered treatment. The third and fourth treatments simulated 'early season cyclical drought' and 'late season cyclical drought', respectively. The early season cyclical drought treatment was applied between October and January (spring/early summer), while the late season cyclical drought treatment was applied between February and May (late summer/autumn). These treatments were timed to coincide with periods of expected early- and latewood development. Trees in these treatments were subjected to a series of drying cycles interrupted by water replenishment when needle water potential had fallen to approximately -0.5 MPa or lower. This water potential was sufficient to generate a depressed growth response as previously observed in non-experimental trees. The early season drought trees were re-watered on November 7, December 1, December 26 and January 16, while the late season drought trees were re-watered on February 20, March 13 and April 19. When not subjected to the cyclical watering treatments between the stated months, water for both treatments was applied at the same times and rates as that for the well watered treatment. The length of time between watering for the cyclical drought treatments depended upon the time of year and air temperature. The time between watering varied from three to seven weeks during the October to May period.

Environmental variables within the polyhouse were measured throughout the duration of the experiment using sensors connected to a data logger (HOBO weather station, ONSET Computer Corporation, Bourne, MA, USA). Air temperature fluctuated between -1 and 35 °C with a mean and standard deviation of 14.3 ± 5.9 °C. Vapour pressure deficit fluctuated between 0.01 and 3.89 kPa with a mean and standard deviation of 0.41 ± 0.53 kPa, while the maximum value of photosynthetically active irradiance was 1023 µmol m^{-2} s^{-1} with a mean and standard deviation of 134 ± 234 µmol m^{-2} s^{-1}.

Predawn needle water potential and measurements of tree growth

Measurements of predawn needle water potential (ψ_e) were made throughout the experiment using a pressure chamber. Three trees per treatment were sampled on each occasion. The timing of sampling depended on the season with weekly sampling occurring in the summer months and fortnightly sampling occurring in the winter months. From each tree, three fascicles were sampled

from the youngest well developed foliage. The fascicles were collected before dawn and stored in test tubes on ice until ψ_e was measured, always within one hour of collection. Following Myers (1988), water stress integral (S_ψ) was determined as the cumulative integral of predawn needle water potential over the period which water deficits were imposed (1 October to 31 May). In addition to total values of S_ψ, monthly S_ψ was also calculated. Values of S_ψ are expressed as an absolute sum, so that larger values of S_ψ represent greater accumulated water deficits.

Measurements of tree growth were taken during the second year that the trees were planted in the polyhouse. Monthly measurements of total tree height and diameter (at a tree height of 0.5 m) were made. Measures of diameter were made on a single axis for all trees using electronic calipers. Stem slenderness was calculated as the ratio of tree height to ground-line diameter. Basal area was calculated using Husch *et al*. (2003).

Aboveground net primary production (ANPP)

Aboveground net primary production was calculated as the sum of aboveground biomass changes (bark, branches, stem and live foliage) and the biomass released as litterfall (woody and foliage litterfall) as:

$$\text{ANPP} = F_A + \Delta C_F + \Delta C_W \quad (1)$$

where F_A is the carbon content of aboveground litterfall, ΔC_F is the change in carbon content of live foliage, and ΔC_W is the change in carbon content of live branches, bark and wood. These can be summarised as foliage NPP ($\Delta C_F + F_A$) and wood NPP (ΔC_W). There was no tree mortality to account for during this experiment.

Final total and component dry masses were determined by harvesting all 48 trees at the end of the experiment and dry masses of second year growth were used to calculate ΔC_F and ΔC_W. Second year growth was determined as the difference between initial and final aboveground mass for the 13 month period that the experimental treatments were applied. Initial mass was determined by destructively sampling eight trees at the start of the second year of growth. There were no statistically significant differences between the eight trees at the start of the experiment for each of the aboveground components (branch, stem and foliage). Consequently, the average component mass of these trees was subtracted from final component mass for each of the 48 experimental trees at the end of the experiment to give second year growth. All tree components were dried at 70 °C to constant mass and weighed. For all calculations, carbon content was assumed

to be 50% of tree dry mass (Vogt, 1991). Litterfall, which was exclusively from needles, was collected monthly, dried and aggregated to yield F_A for each tree.

Total belowground carbon flux (TBCF)

Total belowground carbon fluxes were estimated using a mass balance based approach as described in Giardina and Ryan (2002) and Giardina *et al*. (2003). The method is based on an assumption that all carbon allocated belowground must either be respired, leached or stored. TBCF can be calculated as:

$$\text{TBCF} = F_S + F_E - F_A + \Delta C_S + \Delta C_R + \Delta C_L \quad (2)$$

where F_S is annual soil CO_2 flux, F_E is annual carbon loss from the system by leaching or erosion, F_A is annual litterfall, ΔC_S is the change in carbon content of the mineral soil, ΔC_R is the change in carbon content of root biomass, and ΔC_L is the change in carbon content of the litter layer.

Soil CO_2 efflux was measured monthly for all trees using a soil respiration chamber (100 mm diameter, Model SRC-1, PP Systems, Herts, UK) and an infrared gas analyzer (Model EGM-4, PP Systems, Herts, UK) via a closed system. PVC collars were installed into each of the 48 pots one week prior to the onset of the measurements. All collars were inserted into the soil to a depth of 50 mm to ensure stability and avoid CO_2 lateral diffusion (Davidson *et al*., 2002). Soil temperature was measured using a portable thermometer placed at 100 mm depth. Monthly measurements for all trees were made within the same day. Cumulative soil CO_2 efflux was estimated for each tree using linear interpolations of soil CO_2 flux between measurement dates over the 13 month measurement period (Ryan *et al*., 2010; Epron *et al*., 2012; Nouvellon *et al*., 2012).

In addition to the 48 experimental units, four pots filled with soil but without trees planted, were set aside as controls for measurements of mineral soil respiration. The virgin experimental soil had not been previously subjected to planted trees which had released C in the form of root exudates, biomass turnover or from mycorrhizae, but yet was rich in organic matter, and expected to significantly influence total soil respiration. As such, mineral soil respiration was measured so as to account for non-experimental related sources of carbon respiration. These control pots were subjected to the same regimes of irrigation as those applied in the experimental pots carrying trees (one pot per treatment) and were measured monthly at the same time as the experimental pots. Cumulative mineral soil respiration was estimated using linear interpolation between measurement dates over the 13 month period. The mineral soil respiration values were then subtracted from

values of total soil CO_2 flux (measured above) to give an estimate of annual soil CO_2 flux (F_S) for each tree.

Carbon lost by leaching (F_E) was considered negligible relative to TBCF and was not quantified in this 13 month study, as in similar studies (e.g. Forrester *et al*., 2006; Bown *et al*., 2009; Ryan *et al*., 2010; Nouvellon *et al*., 2012). As the litter layer was non-existent at the start of the experiment and litterfall was collected monthly, values of ΔC_L were set to zero. Litterfall (F_A) was included in the calculation of ANPP but not for TBCF, as there was no addition of litterfall to the soil. Initial and final carbon content of the mineral soil were determined by loss on ignition to yield ΔC_S. Mineral soil carbon concentration at the end of the experiment (6.2%) did not differ significantly from that observed at the beginning of the experiment (6%). Therefore ΔC_S was considered to be negligible.

Values of ΔC_R were determined as the difference between initial and final root mass. Eight trees were destructively sampled at the start of the second year of growth to yield initial root mass. At the conclusion of the second year, final root mass was determined by sampling three trees per treatment (12 trees in total) destructively. The bulk root systems of sampled trees were collected and all visible roots were recovered using a combination of sieving and floatation methods. The bulk root systems were washed to minimise mineral soil contamination. Initial and final root masses were dried at 70 °C to constant mass and weighed. Carbon content was assumed to be 50% of root dry mass (Vogt, 1991). Root biomass for the 36 remaining trees was estimated using an allometric relationship between root biomass and basal area developed from the 12 trees in which root biomass was measured. Basal area provided the best fit from a range of potential predictor variables (r^2 = 0.80). This approach to determining tree or component biomass has been used widely (e.g. Haynes and Gower, 1995; Ryan *et al*., 1996; King *et al*., 1999; McDowell *et al*., 2001; Giardina and Ryan, 2002; Giardina *et al*., 2004; Stape *et al*., 2008). Given the short duration of this study, we assumed that there was little significant root mortality between initial and final estimates of belowground biomass (Vogt and Bloomfield, 1991; Thomas *et al*., 1996; Bown *et al*., 2009).

In summary, values of F_E, F_A, ΔC_S and ΔC_L were assumed to be negligible, so that Equation 2 simplified to:

$$TBCF = F_S + \Delta C_R \quad (3)$$

Partitioning of gross primary production

Gross primary production was computed as the sum of aboveground net primary production (ANPP), total belowground carbon flux (TBCF) and aboveground plant respiration (APR). APR was not

measured directly but was estimated from ANPP, assuming a constant carbon use efficiency (CUE) ratio of 0.5025. This value was reported by Bown *et al*. (2009) examining construction and maintenance respiration of wood and foliage in one-year-old glasshouse grown *P. radiata*. This approach to accounting for APR has been used in numerous studies (e.g. Campoe *et al*., 2012; Epron *et al.,* 2012; Nouvellon *et al*., 2012). NPP was determined from the sum of belowground NPP, estimated above as ΔC_R, and ANPP. For testing of functional partitioning, ΔC_R /ANPP, F_S /ΔC_R and TBCF/ANPP ratios were calculated.

STATISTICAL ANALYSIS

All analyses were carried out using R software (R Core Team, 2013). There were no statistically significant treatment differences in any of the measured variables at the start of the experiment. The effect of water deficits on tree growth, carbon fluxes and carbon partitioning were examined using a mixed effects model that included treatment as a fixed effect with block as a random effect as follows:

$$Y_{ij} = X_i \beta + Z_j b_j + e_{ij} \quad (4)$$

where Y_{ij} is the observation vector of the response variable, X is the fixed effect of treatment, Z is the random effect of block and e is the error term. The subscripts $_i$ and $_j$, represent treatment and block, respectively. β is the vector of fixed effect coefficients, and b is the vector of random effect coefficients. The random effect, b_j, and error, e_{ij}, are independent and normally distributed with mean zero and variances σ_b^2 and σ_e^2, respectively.

Linear models were developed for the early and late season drought treatments when examining the relationship between monthly water stress integral and monthly stem diameter growth using the following form:

$$y = a + bx \quad (5)$$

where y is monthly stem diameter growth, a is the intercept, b is the slope and x is monthly water stress integral. The slopes of the early and late season drought treatments were examined to see if they were significantly different at $P < 0.05$.

Tukey's HSD test was used, where applicable, to distinguish between treatment mean values. Differences were considered significant at $P < 0.05$.

RESULTS

Predawn needle water potential

The implementation of the early season cyclical drought, late season cyclical drought and summer drought treatments had a pronounced effect on ψ_e and induced consistently more negative values of ψ_e than for the well watered treatment (Figure 3.1). The early season and late season cyclical drought treatments resulted in large fluctuations of ψ_e. In both cases, application of water decreased ψ_e immediately and usually, dramatically. The summer drought treatment induced consistently more negative values of ψ_e during the period between mid-December and April. Values of S_ψ were 41.4, 66.8, 55.2 and 97.6 MPa-days for the well watered, early season drought, late season drought and summer drought treatments, respectively.

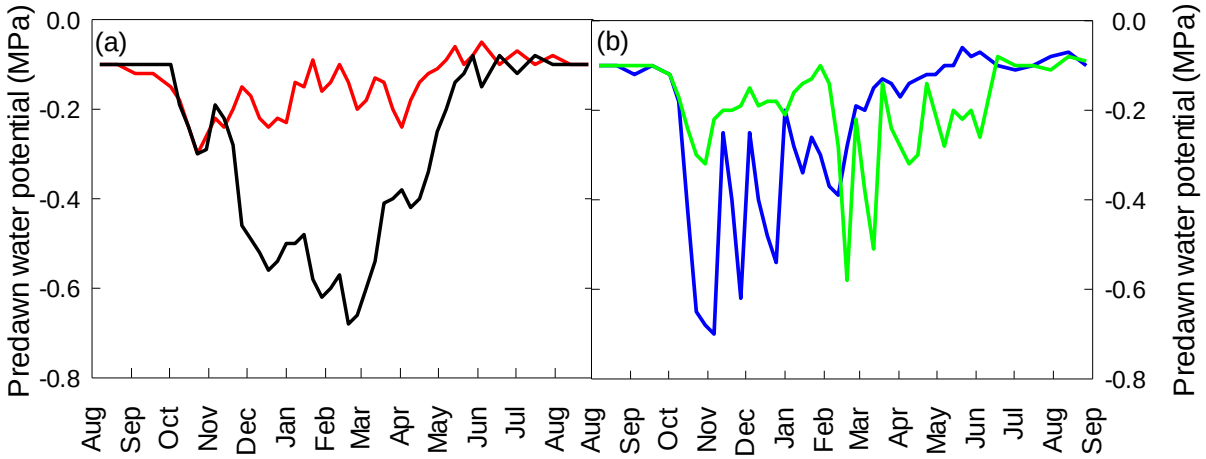


Figure 3.1. Seasonal changes in predawn needle water potential for (a) the well watered (red line) and summer drought (black line) treatments, and (b) early season cyclical drought (blue line) and late season cyclical drought (green line) treatments.

Tree growth

Tree height, diameter at 0.5 m and basal area were all significantly influenced by treatment ($P < 0.001$). Stem slenderness was not influenced by treatment ($P = 0.176$). Basal area was the most sensitive indicator of water deficits (Table 3.1). With the imposition of water deficits, marked divergence in monthly diameter (Figure 3.2) and height growth became immediately apparent. Decreased growth for all water deficit treatments coincided with more negative predawn needle water potential. Compared to the well watered trees, the summer drought treatment reduced height, diameter and basal area by 25.0%, 31.2% and 50.7%, respectively.

Table 3.1. Treatment variation in tree dimensions at the end of the experiment.

Treatment	Tree height (m)	Diameter (mm)	Basal area (mm^2)	Slenderness ($m\ m^{-1}$)
Well watered	3.48 (0.98) a	37.04 (0.96) a	1503 (71) a	79.9 (1.7) a
Early season drought	3.15 (0.60) ab	31.84 (0.62) b	1071 (38) b	85.4 (1.3) a
Late season drought	2.95 (0.80) bc	29.73 (0.46) b	942 (32) b	85.4 (1.8) a
Summer drought	2.61 (0.67) c	25.47 (0.50) c	741 (17) c	85.2 (1.9) a
Analysis of variance Treatment	18.8***	57.5***	58.1***	1.88^{ns}

Each value is the treatment mean with standard error in parentheses for 12 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as *F* value followed by *P* category: ^{ns}non-significant; ***significant at $P < 0.001$.

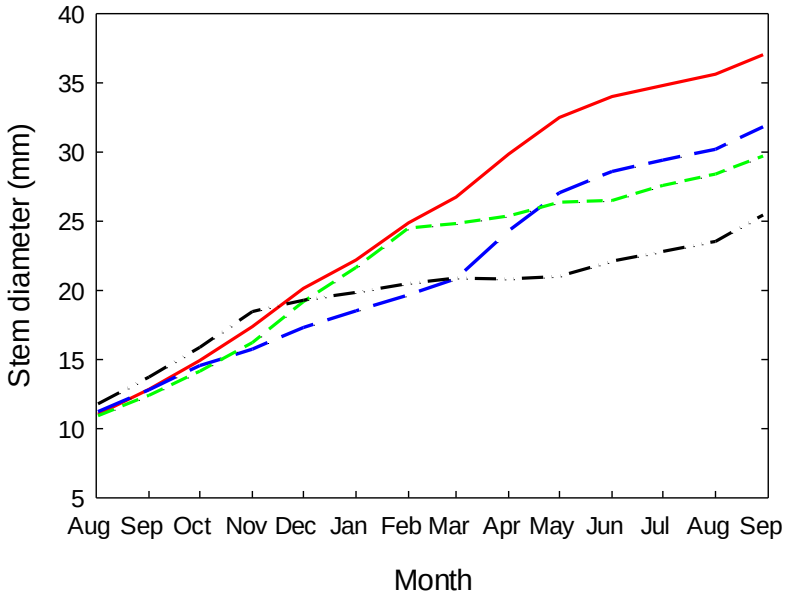


Figure 3.2. Stem diameter growth for the well watered (red line), early season cyclical drought (long blue dash), late season cyclical drought (short green dash) and summer drought (black dash-dot-dot) treatments.

There were no significant differences ($P > 0.05$) in height, diameter and basal area between early and late season drought trees at the end of the experiment (Table 3.1) although total S_ψ was larger in the early season drought trees than in the late season drought trees. Monthly diameter growth was greater in the early season drought trees even though they were subjected to higher levels of water stress than those in the late season drought treatment (Figure 3.3). Diameter growth

was similar for both treatments irrespective of season during those periods when not subjected to water deficits and when monthly S_ψ values were comparable.

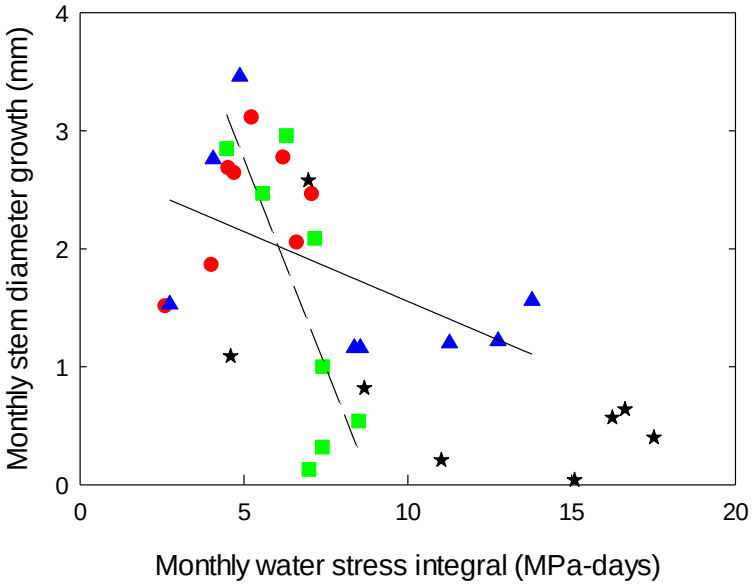


Figure 3.3. The relationship between monthly water stress integral (S_ψ) and monthly stem diameter growth for the period between October 2009 and May 2010 for the well watered (red circles), early season drought (blue triangle), late season drought (green square) and summer drought (black star) treatments. Best fit lines have been included for the early season drought (solid line, r^2 = 0.32) and late season drought (dashed line, r^2 = 0.57) treatments. The slopes were significantly different (P = 0.0399).

Aboveground net primary production

Water deficits significantly influenced carbon fluxes (P < 0.001) to aboveground tree components (Table 3.2). Carbon fluxes to foliage and stem components followed productivity (tree size) gradients with the early season drought treatment exhibiting greater fluxes than the late season drought treatment, despite being subjected to greater water stress. Litterfall was low for all treatments, amounting to less than 4.73 g C tree^{-1}. At the end of the experiment, ANPP of the well watered treatment was over twice that of the summer drought treatment. Foliage NPP and wood NPP of the summer drought treatment was 51.7% and 37.5% of the well watered treatment.

Partitioning of ANPP to foliage and woody components was significantly influenced by treatment (P < 0.001), however, partitioning to woody components decreased following gradients of productivity instead of values of S_ψ. Wood was the dominant component of ANPP for all treatments (51.0 - 59.0%), while litterfall represented less than 0.02% of ANPP for all treatments. The fractions

of GPP allocated to foliage NPP and wood NPP were not affected by treatment and combined, accounted for less than 27.3% of GPP for any treatment.

Table 3.2. Carbon flux and partitioning by treatment.

	Well watered	Early season drought	Late season drought	Summer drought	*Analysis of variance* Treatment
C Fluxes (g)					
ANPP	888.2 (54.9) a	546.4 (30.0) b	514.8 (33.7) bc	384.3 (18.2) c	42.9***
Litterfall (F_A)	2.94 (0.35) b	1.08 (0.16) c	4.73 (0.67) a	4.09 (0.74) a	14.2***
Foliage (ΔC_F)	359.9 (20.3) a	227.4 (11.3) b	217.6 (16.6) b	183.4 (7.8) b	32.7***
Wood (ΔC_W)	525.4 (37.0) a	317.9 (20.7) b	292.57 (17.5) b	196.8 (10.6) c	44.9***
Stem	370.2 (28.1) a	240.5 (14.9) b	209.6 (11.3) b	131.3 (8.3) c	41.1***
Branch	155.2 (12.4) a	77.4 (5.9) b	82.9 (7.9) b	65.5 (3.8) b	32.3***
TBCF	2374.9 (217.7) a	1458.4 (267.1) b	1170.4 (254.1) b	705.8 (101.3) b	12.8***
Soil CO_2 (F_S)	2126.2 (218.6) a	1286.9 (268.0) b	1032.6 (251.4) b	596.7 (101.9) b	10.9***
Roots (ΔC_R)	248.8 (12.9) a	171.4 (7.2) b	137.8 (8.9) bc	109.1 (4.6) c	46.3***
APR (R_A)	879.4 (54.3) a	540.9 (29.7) b	509.8 (33.4) bc	380.5 (18.0) c	42.9***
NPP	1137.0 (67.0) a	717.8 (35.9) b	652.6 (40.5) bc	493.4 (20.3) c	47.6***
GPP	4142.5 (254.6) a	2545.7 (287.0) b	2195.0 (265.2) bc	1470.6 (100.0) c	24.9***
C Partitioning (%)					
ANPP : GPP	21.9 (1.3) a	23.6 (2.0) a	26.0 (2.8) a	27.3 (2.2) a	1.52^{ns}
TBCF : GPP	56.4 (2.5) a	53.0 (3.9) a	48.5 (5.4) a	45.6 (4.4) a	1.51^{ns}
NPP : GPP	28.1 (1.6) a	31.2 (2.7) a	32.9 (3.4) a	35.1 (2.7) a	1.44^{ns}
WNPP : ANPP	59.0 (1.0) a	58.0 (0.7) a	57.1 (0.8) a	51.0 (0.7) b	26.3***
ΔC_R : ANPP	28.2 (0.7) a	31.9 (1.2) a	27.4 (1.6) a	29.0 (1.7) a	1.32^{ns}
F_S : ΔC_R	88.4 (1.4) a	83.8 (2.3) a	78.7 (7.4) a	77.2 (6.0) a	1.26^{ns}
TBCF : ANPP	71.7 (2.2) a	68.3 (3.2) a	63.1 (5.3) a	60.9 (4.6) a	1.64^{ns}

Each value is the treatment mean with standard error in parentheses for 12 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as *F* value followed by *P* category: ^{ns}non-significant; ***significant at $P < 0.001$. WNPP refers to wood NPP.

Soil CO_2 flux

Soil CO_2 flux exhibited strong seasonal variations in response to soil temperature, and additionally for the water stressed treatments, periods of water deficits (Figure 3.4). For the well watered treatment, where water deficits were negligible, a strong response to soil temperature was observed with soil CO_2 flux increasing from a winter low of 1.65 µmol m^{-2} s^{-1} to peak values of 15.80 µmol m^{-2} s^{-1} in summer. The highest mean value of soil CO_2 efflux which occurred in March for the well watered treatment (15.8 ± 1.13 SE µmol m^{-2} s^{-1}), corresponded to the time of greatest diameter

growth for this treatment as it did for the early season drought treatment. Q_{10}, which corresponds to the rate of increase in soil respiration with a 10 ^{o}C increase in soil temperature was 2.12. Soil CO_2 flux was reduced significantly and was different from the well watered treatment when water deficits were imposed. Soil CO_2 flux almost completely ceased in the summer drought treatment during April. Values of soil CO_2 flux returned to levels on par with the well watered treatment when water deficit treatments ceased. Towards the end of the experiment during winter (August), soil CO_2 flux was similar for all treatments irrespective of differences in tree size or root mass between treatments at that time (8.39 ± 0.42 SE µmol m^{-2} s^{-1}).

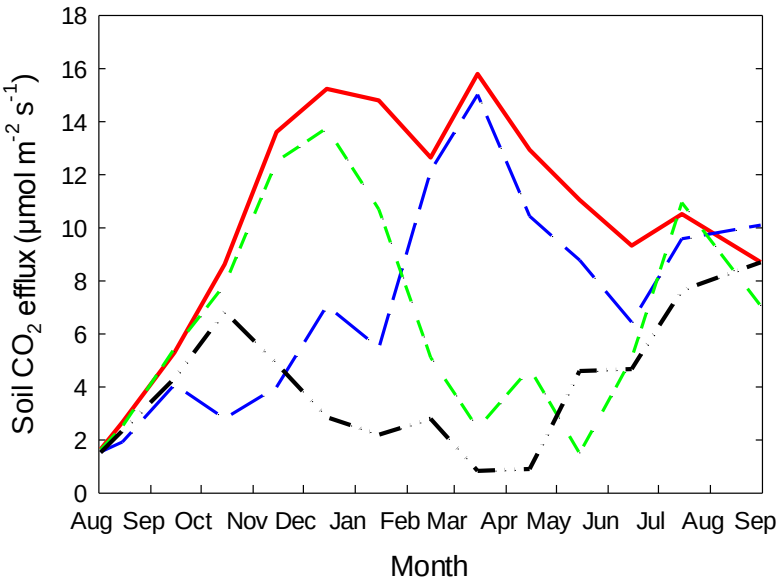


Figure 3.4. Soil CO_2 flux for the well watered (red line), early season cyclical drought (long blue dash), late season cyclical drought (short green dash) and summer drought (black dash-dot-dot) treatments.

Total belowground carbon flux

Total belowground carbon flux by treatment ranged from 706 g C to 2375 g C (Table 3.2). TBCF and fluxes to F_S and root biomass were significantly influenced by treatment ($P < 0.001$). Partitioning of carbon to TBCF was primarily to F_S over root biomass. Partitioning to F_S ranged from 77.2% to 88.4% across the range in treatments, however, there was no significant treatment effects on partitioning of TBCF to F_S. Cumulative F_S was significantly hindered during periods of water deficits and increased with increasing treatment productivity (Figure 3.5). F_S was found to be related positively and significantly to tree diameter ($r^2 = 0.25$, $P < 0.001$). Coarse roots (> 2 mm) contributed more to total

root biomass for the well watered and late season drought treatments (72 and 76%) than for the early season and summer drought treatments (57 and 59%) for those root systems directly sampled.

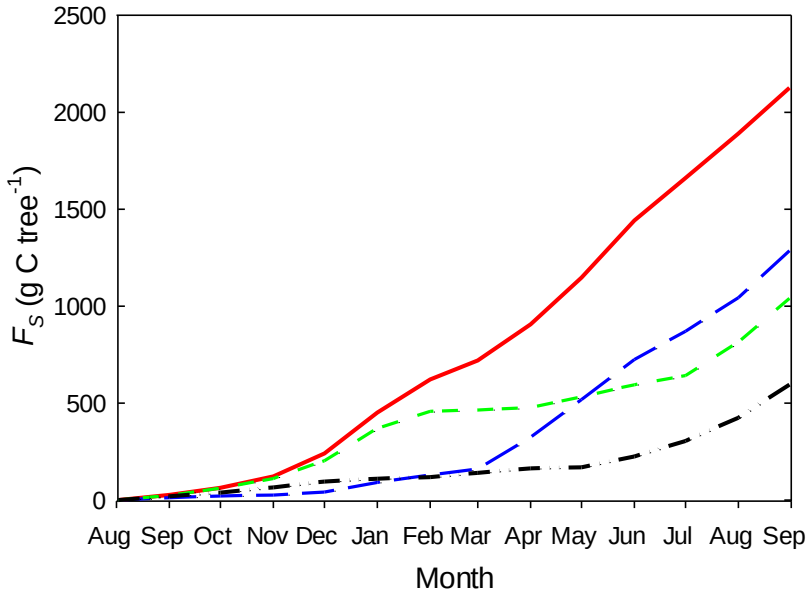


Figure 3.5. Cumulative soil CO_2 flux (F_S) for the well watered (red line), early season cyclical drought (long blue dash), late season cyclical drought (short green dash) and summer drought (black dash-dot-dot) treatments.

Partitioning of gross primary production

GPP ranged from 1470 g C in the summer drought treatment to 4142 g C in the well watered treatment, while NPP ranged from 493 g C to 1137 g C (Table 3.2). Although the early season drought treatment experienced greater levels of water stress, values of NPP and GPP were greater than those for the late season drought treatment, however, these differences were not significant ($P > 0.05$). There was a strong positive relationship between above- and belowground NPP ($r^2 = 0.88$) across all trees.

The fraction of GPP allocated belowground (TBCF/GPP) was approximately equal to that allocated to aboveground components (ANPP and APR) and followed gradients of productivity. This fraction was greatest in the well watered treatment (56.4%) and lowest in the summer drought treatment (45.6%). There were however, no significant differences between treatments ($P > 0.05$) for partitioning of GPP to ANPP or TBCF. The positive relationship between ANPP and TBCF was significant ($r^2 = 0.33$, $P < 0.001$). No significant differences existed between treatments for the F_S /ΔC_R, TBCF/ANPP and ΔC_R/ANPP ratios, with the ΔC_R/ANPP ratio exhibiting a very narrow range in values between treatments (27.4 - 31.9%).

DISCUSSION

A wide range of values for GPP (1470 - 4142 g C) were observed in this experiment in response to the timing and duration of water deficits. In the well watered trees, GPP was 64.5% or 2672 g C $tree^{-1}$ higher than that in summer drought trees. Across all trees in the experiment, there was approximately a five-fold difference in GPP. Fluxes of ANPP were generally, equally allocated to stem wood and foliage components, whilst fluxes of TBCF were preferentially allocated to soil respiration over root production. Fluxes to ANPP and TBCF increased as tree productivity increased and not in response to reduced levels of water stress. There were however, no significant differences in C fluxes between the early and late season drought treatments even though S_ψ was larger in the early season drought trees than in the late season drought trees. This suggests that other factors such as temperature or photosynthetically active irradiance may have been driving or at least exerting a large influence on C production.

Despite large statistically significant differences in carbon fluxes between treatments, partitioning of GPP to ANPP and TBCF was not influenced by treatment. The large variation in water deficits and tree growth between treatments was expected to influence carbon partitioning to above- and belowground components, however, this did not occur. Comparable values of GPP and partitioning responses for juvenile *P. radiata* are unknown because to the best of our knowledge, no previous studies have examined responses to water deficits in a controlled environment. Litton *et al*. (2007), analysing published data representing a wide range of planted and natural forests from tropical to temperate climatic regions, showed a general pattern of increased C partitioned to wood production and less to TBCF with increasing water supply. Similarly, Axelsson and Axelsson (1986) observed increased partitioning to ANPP with increasing water supply in *P. sylvestris*.

Despite the catalogue of studies examining partitioning responses to varying resource availability, it is not known whether mature trees growing in the field respond to varying resource availability with the same phenotypic adjustments as young trees grown under highly controlled conditions (King *et al*., 1999). The lack of any significant differences in partitioning of GPP to ANPP and TBCF between treatments, irrespective of sizeable differences in tree growth within this experiment, suggests that carbon partitioning is probably highly constrained by ontogency (Gedroc *et al*., 1996; King *et al*., 1999). However, imposition of water treatments was only for between four and six months which may have not been enough time for allocation shifts to have taken place before completion of the experiment. Conversely, in a study of mature *P. radiata*, Ryan *et al*. (1996) found partitioning to belowground processes was only marginally different between irrigation and control treatments, while Maier *et al*. (2004) observed a similar result in mid-rotation *P. taeda*, at

least suggesting in some *Pinus* species, that changes in water availability may not influence partitioning of GPP to ANPP and TBCF.

Gross primary production was primarily allocated to TBCF for all treatments and varied between 45.6% and 56.4%, which is higher than values reported by Bown *et al*. (2009) and Ryan *et al*. (1996) for *P. radiata*, but falls inside a range of values reported by Litton *et al*. (2007). Partitioning of GPP to TBCF within the well watered treatment was not statistically different from the summer drought treatment, however, it did represent a greater fraction of GPP, which contradicts numerous studies wherein partitioning increased to ANPP or ANPP components and decreased to TBCF with increasing GPP (Litton *et al*., 2007; Ryan *et al*., 2010; Espinoza *et al*., 2013), often in response to improved resource supply. However, the sizeable range of belowground responses in flux and partitioning to increases in resource supply from a range of studies (Ryan *et al*., 1996; Maier *et al*., 2004; Stape *et al*., 2008) show that no single pattern should be expected to apply generally (Ryan *et al*., 2010).

The high fraction of TBCF partitioned to F_S (77 - 88%) in this study was consistent with that observed by Bown (2007) who found similar fractions (61 - 97%) in young field grown *P. radiata* and Stape *et al*. (2008) who found F_S to be the dominant component of TBCF. Many models of forest carbon budgets have long been simplified by assuming that TBCF is partitioned evenly between root production and respiration (e.g. Ruess *et al*., 1996; Binkley and Ryan, 1998; Waring *et al*., 1998; Law *et al*., 2000; Stape *et al.*, 2004; Newman *et al*., 2006), however, root respiration, carbon losses to root exudates and transfer mycorrhizae can be large fractions of TBCF (e.g. Hobbie, 2006). Typically, such assumptions would introduce significant bias into carbon models. Although we worked with small trees growing in a controlled environment, the highly skewed partitioning of TBCF to F_S in this study demonstrates the potential for errors in making such assumptions for carbon models.

Functional balance theory would suggest that the water deficit treatments should have partitioned larger amounts of C to belowground sinks, however, these findings do not support this. The larger relative amount of C allocated to soil respiration and TBCF by the well watered treatment, and the lower relative amount of C allocated to soil respiration and TBCF by the summer drought treatment meant that the TBCF/ANPP ratio did not increase with water stress, nor did roots of water stressed trees proportionally respire more than roots of non-stressed trees as hypothesized. The summer drought treatment was found to have the same ΔC_R /ANPP ratio as the other treatments, which is generally inconsistent with commonly observed increases in the root to shoot ratio when water availability is limited (Albaugh *et al*., 1998; King *et al*., 1999; Espinoza *et al*., 2013). As hypothesized, the early and late season drought treatments were found to partition similar amounts belowground, although there were no significant differences between any treatments.

Our estimates of APR rely on the assumption that the CUE ratio for aboveground growth is constant among treatments. Use of a constant CUE ratio has been questioned (DeLucia *et al*., 2007), but direct estimations of aboveground CUE are rather scarce and are often complicated by uncertainties due to the cumulative error resulting from scaling chamber based respiration measurements, variations associated with the respiration of different tissues (Waring *et al*., 1998) and use of inferred or estimated values for components that contribute to determining CUE (Epron *et al*., 2012). We used the average aboveground ratio of CUE (0.5025) reported for glasshouse grown *P. radiata* trees one year younger than those in this experiment and subjected to fertilisation treatments (Bown *et al*., 2009), however, we are confident that the estimates of APR are reasonable. The linear interpolations of soil CO_2 efflux assumed that no diurnal differences existed. Soil respiration is strongly affected by temperature (Howard and Howard, 1993; Davidson *et al*., 1998), however, given the setup of this experiment which utilised pot grown trees in a polyhouse, which would have resulted in relatively constant soil temperature diurnally, we assumed no diurnal variability. Consequently, all soil measurements are on a comparative basis and any error would be across all our treatments. Litterfall was omitted from the mass balance TBCF equation since it was collected monthly. Given that litterfall from the young trees over the experiment was very low and accounted for less than 0.01% of GPP for all treatments, whereas, F_S accounted for between 40.5% and 51.3% of GPP, it is unlikely that litterfall removal would have had any impact on F_S or the interpretation of F_S/TBCF fractions in this study.

The production ecology of a single *P. radiata* clone grown in an artificial environment may not represent the environmental responses of other *P. radiata* genotypes on a range of sites, however, this study shows how one particular *P. radiata* genotype responded to unique water deficit conditions. The polyhouse grown trees allowed us to employ water deficit treatments that would have otherwise been unfeasible to emulate in the field, while analysing water deficit effects on individual tree carbon fluxes and partitioning trends, something to the best of our understanding, has not been attempted before. The polyhouse grown trees are undoubtedly different to field grown trees, therefore we are cautious about extrapolating results. Extrapolating results of juvenile trees, grown either in the field or controlled conditions is also difficult, as different phenological and physiological processes occur in mature trees, and the timing, duration and location within the tree of these processes changes as well. Nonetheless, we believe the results obtained provide valuable insights into the processes displayed by juvenile *P. radiata*.

CONCLUSIONS

Water deficits significantly influenced absolute values of carbon fluxes to ANPP, TBCF, NPP and GPP ($P < 0.001$). The observed increase in ANPP, TBCF, NPP and GPP fluxes from low to high productivity classes could not be explained solely by the increase in resource availability. Treatment values of GPP were between 1470 and 4142 g C per tree. There were no significant treatment differences ($P > 0.05$) in partitioning of GPP to ANPP and TBCF and partitioning of GPP was predominantly to TBCF (45 - 56%) for all treatments. Soil respiration (F_S) was the dominant component of TBCF (77 - 88%) for all treatments. As hypothesized, the early and late season drought treatments were found to partition similar amounts belowground (53.0 and 48.5%, respectively). The larger relative amount of C allocated to soil respiration and TBCF by the well watered treatment meant that the TBCF/ANPP ratio did not increase with water stress, nor did roots of water stressed trees proportionally respire more than roots of non-stressed trees as hypothesized. Results suggest that water deficits are more important in delaying growth than in changing patterns of carbon allocation in *P. radiata*.

CHAPTER FOUR

INFLUENCE OF WATER DEFICITS ON WOOD DENSITY, MICROFIBRIL ANGLE, FIBRE PROPERTIES AND MODULUS OF ELASTICITY OF JUVENILE *PINUS RADIATA*

SUMMARY

Cambial growth and wood properties respond to fluctuating environmental conditions. Understanding the nature of these responses is crucial to understanding their cumulative effects on wood quality characteristics of a forest stand. This experiment examined the responses of tree growth and wood properties in juvenile *Pinus radiata* D. Don to the timing and duration of water deficits over the course of one year. The treatments were well watered, early season cyclical drought, late season cyclical drought and extended summer drought. At the conclusion of the experiment, wood samples were analysed by X-ray absorption, X-ray diffraction and automated image analysis using Silviscan. Wood properties were rescaled from a distance scale to a time scale using monthly measures of tree diameter. At a seasonal level, juvenile *Pinus radiata* wood properties were sensitive to temporal changes in water availability and associated growth rates. Imposition of seasonal water deficits resulted in higher values of density and modulus of elasticity (*E*) and decreases in microfibril angle (MFA). These differences were more evident for the late season drought treatment than for the early season drought treatment when compared to the well watered treatment. Late season drought increased density by 10%, *E* by 18% and decreased MFA by 5% compared with the well watered trees. Seasonal water deficits had no impact on annual average values of density, *E* and MFA in this experiment but significant annual differences existed in cell wall thickness, cell radial diameter and cell population between the faster and slower growing trees. The results highlight the direct effects of water availability on wood properties and the need for forest managers to consider management strategies that balance productivity with wood quality for timber production.

INTRODUCTION

Advances in tree breeding and changes in silvicultural practice over the last few decades have greatly enhanced growth rates of plantation-grown conifers. These growth gains have led to shorter rotations and an increased proportion of corewood (Cown, 1992). Physical and mechanical properties of corewood differ from those of mature wood (DeBell *et al*., 2002) and it is widely accepted that coniferous trees grown in plantation conditions have corewood with physical features that are inferior to those found further from the pith (Butterfield, 2003). Corewood is generally characterised by low density, thin cell walls, short fibres with small lumens, high grain angle and high microfibril angle (MFA), with the result that it has low strength and stiffness and poor dimensional stability compared to mature wood (Macdonald and Hubert, 2002).

In the widely grown plantation softwood *Pinus radiata* D. Don, one of the most important mechanical properties for solid timber applications is modulus of elasticity (*E*), more commonly known as stiffness (Yang and Evans, 2003). This property, which is used to define elastic behaviour of wood, measures the resistance of wood to deflection under an applied load and is used as a criterion in machine stress grading of structural timber (Walker and Nakada, 1999). It is also required for determining quality of laminated veneer lumber. *E* is often considered more important than strength for predicting wood quality because *Pinus radiata* boards rarely break in normal use; much more frequently a load results in excessive deflection (Walford, 1985). The widespread adoption of machine stress grading of timber has highlighted both the generally substandard nature of the resource and the gains that would be possible if *E* in juvenile wood could be increased sufficiently to reach thresholds for structural grade (Walker and Nakada, 1999).

Microfibril angle (MFA) and wood density are the two primary wood properties influencing *E* and the subsequent quality of solid wood products (Cave and Walker, 1994). High values of *E* are the result of low MFA and high wood density. Microfibril angle is the angle of the cellulose microfibrils in the cell wall relative to the long axis of the cell. The dominant layer of the cell wall which determines the axial stiffness and the strength of the wood is the S2 layer which occupies about 80% of the volume of the cell wall. High values of MFA are found in corewood, and within annual growth rings, higher values of MFA are found in earlywood than latewood (McMillin, 1973; Donaldson, 1992). As the other main determinant of *E*, wood density is affected by a range of fibre characteristics including cell wall thickness and cell diameter, as well as the earlywood to latewood ratio within the annual ring (Cave and Walker, 1994). Density typically increases with increasing ring number from the pith (Walker and Nakada, 1999).

Past research has shown that in *P. radiata*, corewood properties can be manipulated by management strategies. Significant gains in important wood properties such as air-dried density, MFA and *E* at single sites have been shown through increasing initial stand density (Lasserre *et al*., 2009), reducing the level of woody weed control (Watt *et al*., 2005) and using clones with improved wood properties (Lindstrom *et al*., 2004). Significant variation in wood properties has also been shown between sites with properties improving in warmer areas (Watt *et al*., 2006). However, despite an improved understanding of how environment influences corewood properties (Watt *et al*., 2009), high resolution, intra-ring information on the response of corewood properties to seasonal water deficits is lacking.

Pinus radiata is likely to be subjected to increasingly erratic seasonal rainfall, more frequent and severe drought, and increased evaporative demand brought about by higher temperatures under predicted climate change in decades to come (Kirschbaum and Fischlin, 1996; Kirschbaum *et al*., 2012). At present, soil water deficits are common across many eastern regions of New Zealand during summer (Palmer *et al*., 2009) and many of these areas are predicted to receive less rainfall in the future[4] with an increased risk of severe drought (Mullan *et al*., 2005). Forest managers can also influence soil water availability through choices in site, stand density and level of weed control (Nambiar, 1990; Stogsdili *et al*., 1992; Yunusa *et al*., 1995*a*; Watt *et al*., 2003; Watt *et al*., 2006).

Wood formation in trees is a dynamic process that is strongly affected by environmental conditions (Antonova and Stasova, 1997; Deslauriers and Morin, 2005; Rossi *et al*., 2006). Water deficits can affect wood formation, directly, through effects on cambial cells and their derivatives or, indirectly, through effects on photosynthesis and the translocation of assimilates from the crown (Denne and Dodd, 1981; Abe *et al*., 2003; Arend and Fromm, 2007). Periods of drought affect wood properties by reducing xylem cell production, fibre diameter and cell wall thickness (Whitehead and Jarvis, 1981) and hence, properties such as wood density and MFA may be markedly altered (Drew *et al*., 2009). Given that wood property variation is at its largest at the sub-annual level in trees growing in seasonal climates (Labosky and Ifju, 1972), any variation in water availability during the growing season is likely to affect wood formation. Although past research over sub-annual intervals has reported the importance of water availability in regulating density, MFA and *E* (Wimmer *et al*., 2002*a*; Wimmer *et al*., 2002*b*; Watt *et al*., 2005), little is known about the influence of timing and duration of drought on density, MFA, fibre properties and *E* in very young *P. radiata* wood.

A better understanding of the effect of water availability on wood properties is crucial, as this will enable forest managers to closely match sites with correct genetic material and silvicultural

[4] http://www.mfe.govt.nz/publications/climate/climate-change-effect-impacts-assessments-may08/page4.html

regimes to optimise growth and product outturn. The objective of the experiment was to elucidate the effects of water availability mediated through the timing and duration of drought, on trends in density, MFA, fibre dimensions and *E* in corewood of juvenile *P. radiata* by matching seasonal variation in growth, measured temporally, with variation in wood properties, measured spatially.

METHODS

Experimental design and treatments

The experiment was undertaken in a polyhouse which provided growing conditions where water supply to *Pinus radiata* D. Don clonal stock could be controlled. This plant material was a high wood density, high acoustic velocity clone (Clone 35, Forest Genetics CellFor Ltd) produced using somatic embryogenesis. The nursery raised trees were planted in the polyhouse for a two year period and the experimental treatments were applied during the second year. The trees were planted in a free draining loamy sand soil in 200 litre pots to reduce any potential for water logging and root binding. Soil texture and particle size were determined prior to experiment commencement using a hydrometer for smaller particles and sieve for larger particles. Particles were separated into <0.002, 0.002-0.06, 0.06-0.2, 0.2-0.6 and 0.6-2.0 mm size classes. Medium and coarse sand compromised 76% of the soil.

The clonal tree material grown in the polyhouse was subjected to four water deficit treatments laid out in six blocks of five rows for one growing season (August 2009 to September 2010). Within each block of five rows, all four water deficit treatments were present plus one buffer row. Each treatment, which occupied one row, consisted of four trees. The treatments within each block were assigned randomly. The buffer row within each block was placed systematically on the next row to the north of rows subject to the summer drought treatment so that shading of the drought trees and thus any confounding influences were minimised. Trees were spaced 1.4 m apart so that there was no physical interaction between their crowns. Twenty four trees per treatment were present giving a total of 96 trees.

Four water deficit treatments were applied to the experimental trees. Irrigation was applied using drip sprinklers until pre-specified predawn needle water potentials were reached. The first treatment was ‘well watered’ with applications of water applied weekly between October and May (spring to autumn), and fortnightly between June and September (winter). The second treatment simulated ‘extended summer drought’ whereby water was withheld between November and April to allow drying of the soil profile. The drought was interrupted by the small, single application of water

on March 1 to prevent tree mortality. Although predawn needle water potentials were not particularly low at this time, the trees had started showing large physical changes to continued drought. During the remainder of the year, water was applied at the same times and rates as the well watered treatment. The third and fourth treatments simulated 'early season cyclical drought' and 'late season cyclical drought', respectively. The early season cyclical drought treatment was applied between October and January (spring/early summer), while the late season cyclical drought treatment was applied between February and May (late summer/autumn). These treatments were timed to coincide with periods of expected early- and latewood development. Trees in these treatments were subjected to a series of drying cycles interrupted by water replenishment when needle water potential had fallen to approximately -0.5 MPa or lower. This water potential was sufficient to generate a depressed growth response as previously observed in non-experimental trees. The early season drought trees were re-watered on November 7, December 1, December 26 and January 16, while the late season drought trees were re-watered on February 20, March 13 and April 19. When not subjected to the cyclical watering treatments between the stated months, water for both treatments was applied at the same times and rates as that for the well watered treatment. The length of time between watering for the cyclical drought treatments depended upon the time of year and air temperature. The time between watering varied from three to seven weeks during the October to May period.

Environmental variables within the polyhouse were measured throughout the duration of the experiment using sensors connected to a data logger (HOBO weather station, ONSET Computer Corporation, Bourne, MA, USA). Air temperature fluctuated between -1 and 35 °C with a mean and standard deviation of 14.3 ± 5.9 °C. Monthly measures of soil temperature closely corresponded to mean air temperature throughout the course of the experiment (data not shown). Vapour pressure deficit fluctuated between 0.01 and 3.89 kPa with a mean and standard deviation of 0.41 ± 0.53 kPa, while the maximum value of photosynthetically active irradiance was 1023 µmol m^{-2} s^{-1} with a mean and standard deviation of 134 ± 234 µmol m^{-2} s^{-1}.

Predawn needle water potential and measurements of tree growth

Measurements of predawn needle water potential (ψ_e) were made throughout the experiment using a pressure chamber. Three trees per treatment were sampled on each occasion. The timing of sampling depended on the season with weekly sampling occurring in the summer months and fortnightly sampling occurring in the winter months. From each tree, three fascicles were sampled from the youngest well developed foliage. The fascicles were collected before dawn and stored in

test tubes on ice until ψ_e was measured, always within one hour of collection. Following Myers (1988), water stress integral (S_ψ) was determined as the cumulative integral of predawn needle water potential over the period which water deficits were imposed (1 October to 31 May). Values of S_ψ are expressed as an absolute sum, so that larger values of S_ψ represent greater accumulated water deficits.

Measurements of tree growth were taken during the second year that the trees were planted in the polyhouse. Monthly measurements of total tree height and diameter (at a tree height of 0.5 m) were made. Measures of diameter were made on a single axis for all trees using electronic calipers. Stem slenderness was calculated as the ratio of tree height to ground-line diameter. Basal area was calculated using Husch *et al*. (2003). Measures of bark thickness were made at the start of the experiment on eight destructively sampled trees and at the end of the experiment on all trees under observation.

Wood properties data

At the conclusion of the experiment all 96 trees were destructively sampled. From each tree, 30 mm discs were taken at the point of monthly diameter measures (height of 0.5 m). In order to prevent checking and minimise dimensional changes, the water in the disc samples was replaced with 100% ethanol. Four ethanol exchanges were undertaken, with each exchange lasting two days. After the final exchange, the samples were air-dried to constant weight. At this point, a bark to bark slice centred on the pith was sawn from each disc sample and split into two pith to bark radial samples so that the transverse surface was a strip 2 mm wide. Samples were then conditioned at 20 °C and 40% relative humidity.

Radial profiles of air-dried density[5], microfibril angle, fibre dimensions and *E* were estimated using SilviScan at a resolution of 25 µm for density and fibre dimensions and 200 µm for microfibril angle and *E*. SilviScan uses X-ray absorption to directly measure density and X-ray diffraction to estimate microfibril angle. Cell radial diameters are measured using automated image analysis. Cell wall thickness is derived from the cell diameter and density information. *E* is estimated as the product of density and the coefficient of variation of the 002 azimuthal intensity profile from X-ray diffractometry through the radial cell walls (Evans *et al*., 1995; Evans *et al*., 1999; Evans, 2006). Densitometry data was used to identify the annual growth rings and latewood percentage was calculated using a threshold value of 400 kg m^{-3}. Latewood duration was estimated as the number of days when density was greater than 400 kg m^{-3}.

[5] All further references to Silviscan density from this point onwards refer to air-dried density.

For each wood property, distance from the pith was converted to a time during the year of the experiment. This was done by directly matching distance from the pith to measures of diameter taken during the experiment. Wood lost from the pith area during Silviscan sample preparation was accounted for. Bark thickness at the start and end of the experiment was also accounted for and was used to provide a sliding scale of bark thickness over the 13 months of the experiment. Distances from pith for elliptically shaped trees were weighted towards the larger side of the tree. The elliptical shape was assumed to be constant throughout the experiment. The process proved to be very accurate as demonstrated by the very high degree of agreement between the time when water was applied to samples and the subsequent changes in wood properties observed as a result of watering.

STATISTICAL ANALYSIS

All analyses were carried out using R software (R Core Team, 2013). There were no statistically significant treatment differences in any of the measured variables at the start of the experiment. The effect of water deficits on tree growth and wood properties were examined using a mixed effects model that included treatment as a fixed effect with block as a random effect at both the seasonal and annual levels, as follows:

$$Y_{ij} = X_i \beta + Z_j b_j + e_{ij} \quad (1)$$

where Y_{ij} is the observation vector of the response variable, X is the fixed effect of treatment, Z is the random effect of block and e is the error term. The subscripts $_i$ and $_j$, represent treatment and block, respectively. β is the vector of fixed effect coefficients, and b is the vector of random effect coefficients. The random effect, b_j, and error, e_{ij}, are independent and normally distributed with mean zero and variances σ_b^2 and σ_e^2, respectively. Response variables included tree dimensions, density, cell wall thickness, cell radial diameter, microfibril angle, modulus of elasticity, cell population, ring width and latewood percentage.

Spearman's correlation analysis was performed to assess the relationship between monthly growth and wood properties using treatment pooled data. Tukey's HSD test was used, where applicable, to distinguish between treatment mean values. Differences were considered significant at $P < 0.05$.

RESULTS

Predawn needle water potential

The implementation of the early season cyclical drought, late season cyclical drought and summer drought treatments had a pronounced effect on ψ_e and induced consistently more negative values of ψ_e than for the well watered trees (Figure 4.1). The early season and late season cyclical drought treatments resulted in large fluctuations of ψ_e. In both cases, application of water decreased ψ_e immediately and usually, dramatically. The summer drought treatment induced consistently more negative values of ψ_e during the period between mid-December and April. Values of S_ψ were 41.4, 66.8, 55.2 and 97.6 MPa-days for the well watered, early season drought, late season drought and summer drought treatments, respectively.

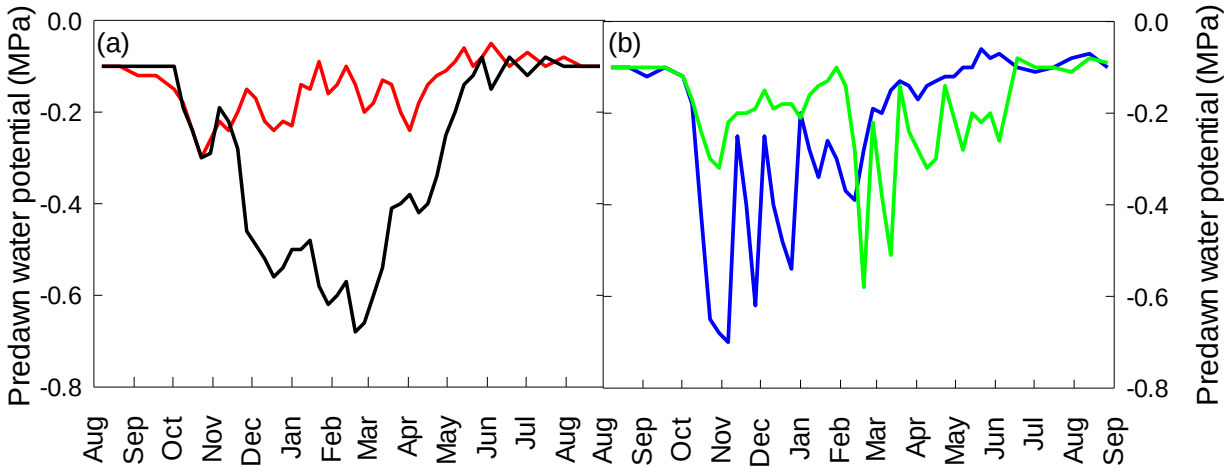


Figure 4.1. Seasonal changes in predawn needle water potential for (a) the well watered (red line) and summer drought (black line) treatments, and (b) early season cyclical drought (blue line) and late season cyclical drought (green line) treatments.

Tree characteristics

Tree height, diameter at 0.5 m and basal area were all significantly influenced by treatment ($P < 0.001$). Stem slenderness was also significantly influenced by treatment ($P = 0.012$), but only due to the variation in slenderness between the well watered and early season drought treatments. Basal area was the most sensitive indicator of water deficits (Table 4.1). With the imposition of water deficits, marked divergence in monthly diameter (Figure 4.2) and height growth became immediately apparent. Decreased growth for all water deficit treatments coincided with more negative predawn needle water potential values. There were no significant differences ($P > 0.05$) in

tree dimensions between early and late season drought trees at the end of the experiment (Table 4.1) although S_ψ was larger in the early season drought trees than in the late season drought trees. Compared to the well watered treatment, the summer drought treatment reduced height, diameter and basal area by 24.7%, 33.1% and 52.3%, respectively.

Table 4.1. Treatment variation in tree dimensions at the end of the experiment.

Treatment	Tree height (m)	Diameter (mm)	Basal area (mm^2)	Slenderness ($m\ m^{-1}$)
Well watered	3.54 (0.55) a	38.26 (0.80) a	1590 (76) a	79.6 (1.2) a
Early season drought	3.22 (0.53) b	32.37 (0.59) b	1086 (35) b	87.0 (1.0) b
Late season drought	2.99 (0.59) b	30.61 (0.58) b	1015 (38) b	83.8 (1.3) ab
Summer drought	2.61 (0.42) c	25.59 (0.42) c	759 (21) c	84.1 (1.2) ab
Analysis of variance				
Treatment	37.3***	44.2***	34.2***	5.12*

Each value is the treatment mean with standard error in parentheses for 24 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as F value followed by P category: *significant at $P < 0.05$; ***significant at $P < 0.001$.

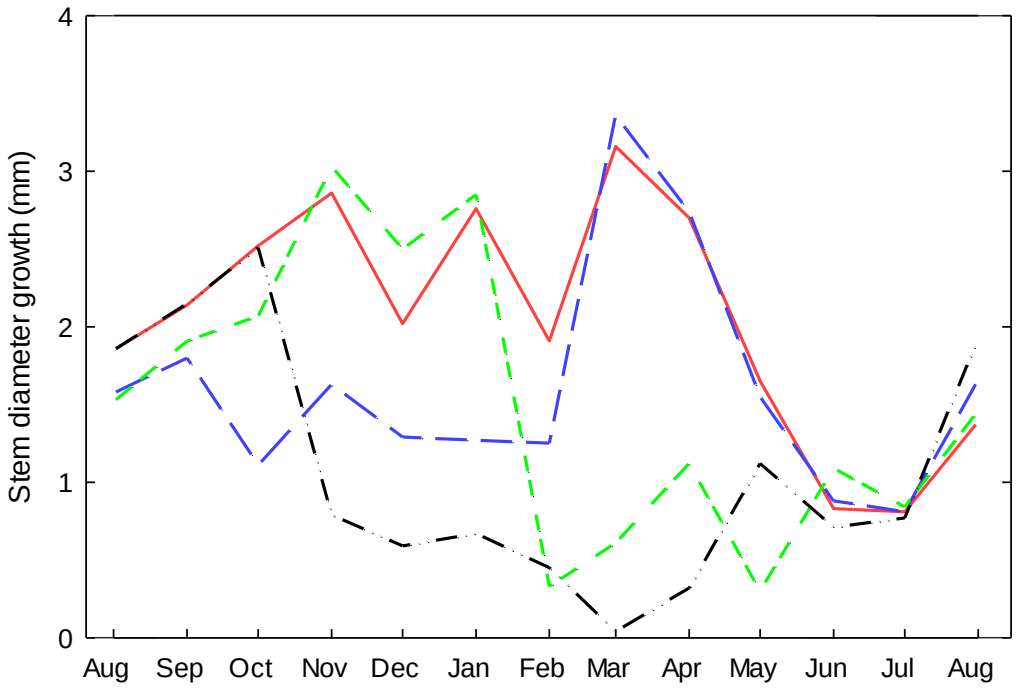


Figure 4.2. Average monthly incremental stem diameter growth for the well watered (solid red line), early season cyclical drought (long blue dash), late season cyclical drought (short green dash) and summer drought (black dash-dot-dot) treatments.

During the period of imposed water deficits for the early season drought between October and January, trees increased in diameter by 5.36 mm compared to 10.60 mm for the late season drought trees and 10.17 mm for the well watered trees. When water deficits were imposed for the late season drought trees between February and May, diameter increased by 2.37 mm compared to 8.90 mm for the early season drought and 9.41 mm for the well treatment trees. Figure 4.2 demonstrates that the reduction in stem diameter growth was more marked for the late season treatment than the early season treatment when compared with the well watered trees. This suggests that late season drought was more detrimental to tree growth than early season drought. The summer drought trees increased in diameter by 2.85 mm between November and the end of April compared with the well watered trees which increased by 15.41 mm.

Wood properties

Significant variation in seasonal water availability and growth rate had little effect on annual average wood properties. Annual average values of density, MFA and *E* were not significantly influenced by treatment (Table 4.2), however, cell wall thickness, cell radial diameter and cell population were ($P < 0.05$). When averaged across the year, between treatment variation was 2.5% for density, 5.2% for cell wall thickness, 5.3% for cell radial diameter, 2.2% for MFA, 4.8% for *E* and 7.2% for cell population. Latewood percentage was significantly influenced by treatment ($P < 0.05$) and large between treatment variation was observed for latewood duration. Late season and summer drought

Table 4.2. Treatment variation in annual average values for wood properties.

Treatment	Density (kg m^{-3})	Cell wall thickness (µm)	Cell radial diameter (µm)	Microfibril angle (degrees)	Modulus of elasticity (GPa)	Cell population (mm^{-2})
Well watered	405 (1) a	1.94 (0.01) a	28.55 (0.02) a	31.42 (0.02) a	5.22 (0.01) a	1411 (2) b
Early season	408 (1) a	1.92 (0.01) a	28.09 (0.03) ab	31.75 (0.02) a	5.10 (0.01) a	1471 (2) ab
Late season	398 (1) a	1.84 (0.01) b	27.05 (0.03) c	31.30 (0.02) a	4.97 (0.01) a	1518 (2) a
Summer	398 (1) a	1.84 (0.01) b	27.38 (0.04) bc	31.06 (0.02) a	5.03 (0.02) a	1520 (3) a
Analysis of variance						
Treatment	1.84^{ns}	20.6***	7.84***	0.64^{ns}	0.35^{ns}	5.65**

Each value is the treatment mean with standard error in parentheses for 24 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as *F* value followed by *P* category: ^{ns}non-significant; **significant at $P < 0.01$; ***significant at $P < 0.001$.

treatments had the effect of increasing latewood duration, whilst early season drought which grew rapidly post treatment, had a significantly delayed onset of latewood formation which subsequently resulted in a lower latewood percentage. The initiation date of latewood varied considerably between treatment, however, it concluded on approximately the same date for all treatments (Table 4.3).

Table 4.3. Treatment variation in ring width and latewood formation.

Treatment	Ring width (mm)	Latewood percentage (%)	Number of latewood days	Latewood start date	Latewood finish date
Well watered	25.87 (0.80) a	18.40 (1.32) b	165	1 March	12 August
Early season	20.24 (0.50) b	15.19 (0.63) b	98	1 May	6 August
Late season	19.08 (0.53) b	17.33 (1.56) b	175	16 February	9 August
Summer	13.23 (0.43) c	28.07 (1.13) a	222	30 December	8 August
Analysis of variance					
Treatment	48.7***	41.1***	65.1***		

Each value for ring width and latewood percentage is the treatment mean with standard error in parentheses for 24 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as F value followed by P category: ***significant at $P < 0.001$. Number of latewood days is the duration between the latewood start and finish dates. Ring width is the year 2 cambium growth and excludes bark.

Seasonal changes in wood properties were strongly influenced by treatment (Figure 4.3; Table 4.4). Compared to the well watered treatment, the implementation of the early season, late season and summer drought treatments resulted in increased values for density, cell population and *E*, and decreased values for cell radial diameter and MFA. Treatment divergence was greatest for *E*, whereby seasonal drought increased values of *E* over the well watered treatment by 11.4%, 17.5% and 19.5% for the early season, late season and summer drought treatments, respectively. Over the course of the year, the early season drought treatment exhibited the smallest deviation from the "normal" well watered treatment for patterns of wood properties. During the period post treatment, the seasonal drought treatments all experienced declines in absolute values of density, cell wall thickness and *E*, whilst experiencing increases in MFA (Figure 4.3) relative to the well watered treatment. However, these differences were typically not significantly different (Table 4.5).

Strong interactions between treatment and time were evident, particularly for density, cell wall thickness and *E*. Minimum values for these wood properties were reached during spring and maximum during winter. Values for these properties were comparable between the start and end of

the experiment in August 2009 and 2010 for all treatments. MFA exhibited a general decline in values for all treatments during the course of the year. Values of *E* exhibited the greatest degree of change for all treatments, increasing three-fold for the well watered treatment from values of 3.2 GPa in spring to values of 9.85 GPa during early winter.

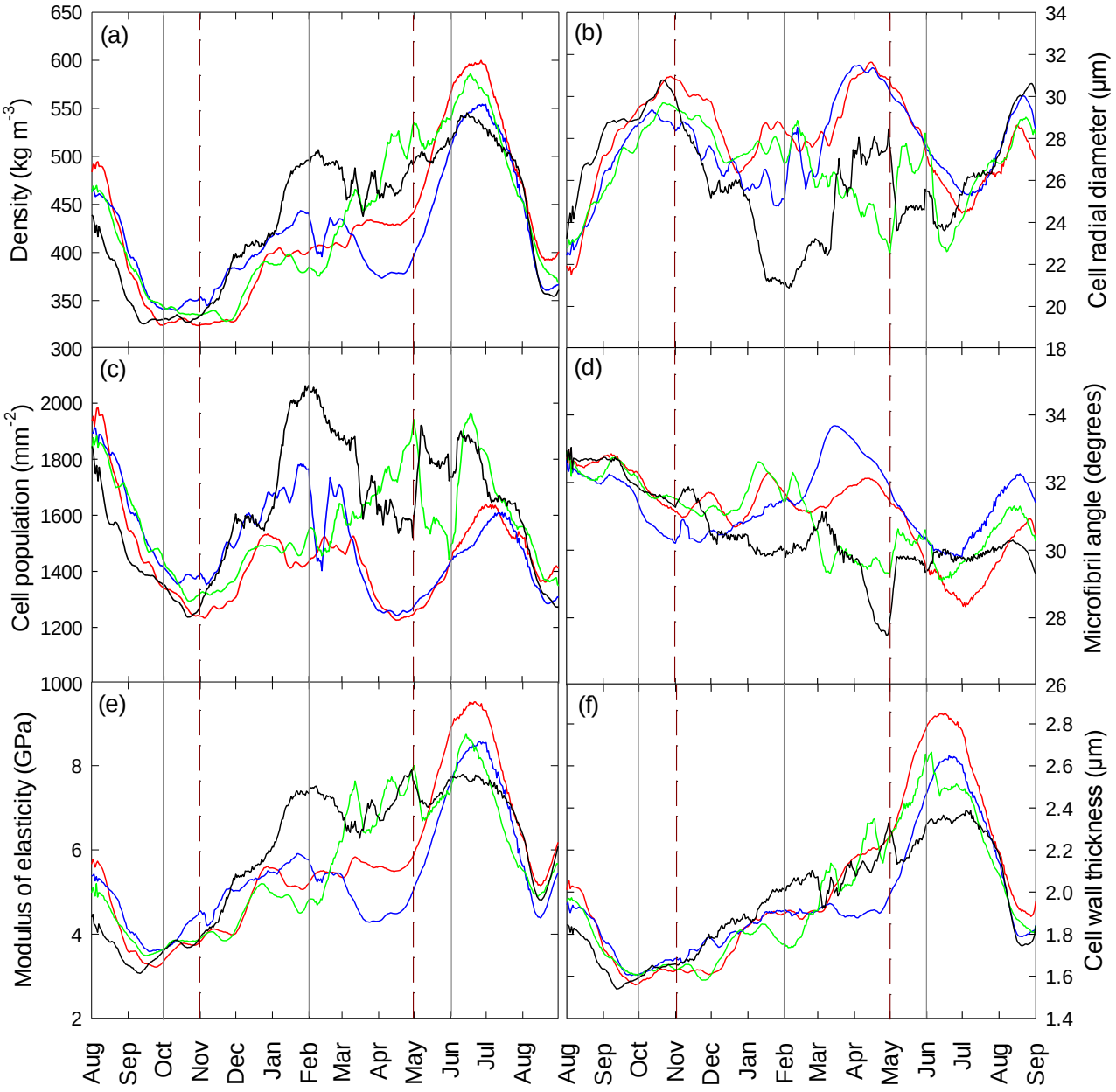


Figure 4.3. Annual changes in (a) density, (b) cell radial diameter, (c) cell population, (d) microfibril angle, (e) modulus of elasticity and (f) cell wall thickness for trees growing in the well watered (red line), early season drought (blue line), late season drought (green line) and summer drought (black line) treatments. The early season (Oct - Jan) and late season (Feb - May) drought treatment periods are indicated by solid vertical lines while the summer drought (Nov - Apr) treatment period is indicated by dashed lines. Data has been averaged using a resolution of 7 days.

Table 4.4. Comparison of wood properties between well watered treatment and the early season (October - January), late season (February - May) and summer drought (November - April) treatments during period of imposed water deficits.

Treatment	Density (kg m^{-3})	Cell wall thickness (µm)	Cell radial diameter (µm)	Microfibril angle (degrees)	Modulus of elasticity (GPa)	Cell population (mm^{-2})
Early season						
Well watered	356 (1) b	1.72 (0.01) b	29.09 (0.03) a	31.46 (0.03) a	4.49 (0.01) b	1362 (2) b
Early season	387(1) a	1.77 (0.01) a	27.17 (0.06) b	30.69 (0.03) a	5.00 (0.02) a	1536 (5) a
AOV	26.6**	7.26*	25.3**	2.74^{ns}	13.4*	35.7**
Late season						
Well watered	439 (1) b	2.16 (0.01) a	29.41 (0.04) a	31.42 (0.03) a	5.94 (0.02) b	1367 (3) b
Late season	482 (2) a	2.14 (0.01) a	24.80 (0.09) b	29.94 (0.05) b	6.98 (0.04) a	1727 (8) a
AOV	8.11*	0.74^{ns}	50.7***	10.3*	55.1***	59.5***
Summer drought						
Well watered	395 (1) b	1.91 (0.01) a	29.11 (0.03) a	31.54 (0.02) a	5.17 (0.01) b	1379 (2) b
Summer	437 (2) a	1.92 (0.01) a	24.78 (0.09) b	30.33 (0.05) a	6.18 (0.04) a	1706 (9) a
AOV	38.3**	0.03^{ns}	39.4**	4.13^{ns}	16.8**	33.6**

Each value is the treatment mean with standard error in parentheses for 24 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as *F* value followed by *P* category: ^{ns}non-significant; *significant at $P < 0.05$; **significant at $P < 0.01$; ***significant at $P < 0.001$. *AOV* is analysis of variance.

Table 4.5. Comparison of post treatment variation in wood properties between well watered treatment and seasonal drought treatments for 3 months after conclusion of imposed water deficits.

Treatment	Density (kg m^{-3})	Cell wall thickness (µm)	Cell radial diameter (µm)	Microfibril angle (degrees)	Modulus of elasticity (GPa)	Cell population (mm^{-2})
Early season						
Well watered	424 (1) a	2.07 (0.01) a	29.52 (0.04) a	31.62 (0.03) a	5.59 (0.01) a	1373 (3) a
Early season	398 (1) b	1.91 (0.01) b	29.73 (0.04) a	32.85 (0.03) a	4.72 (0.01) b	1393 (4) a
AOV	12.6*	75.0***	0.21^{ns}	4.67^{ns}	14.0*	0.18^{ns}
Late season						
Well watered	489 (2) a	2.32 (0.01) a	26.57 (0.07) a	29.66 (0.05) a	7.14 (0.05) a	1492 (5) b
Late season	475 (2) a	2.17 (0.01) b	26.23 (0.09) a	30.26 (0.05) a	6.49 (0.04) a	1613 (8) a
AOV	3.84^{ns}	18.9**	1.13^{ns}	1.41^{ns}	5.13^{ns}	12.8*
Summer drought						
Well watered	523 (2) a	2.60 (0.01) a	27.32 (0.06) a	29.76 (0.06) a	7.98 (0.04) a	1448 (5) b
Summer	514 (2) a	2.27 (0.01) b	24.94 (0.10) b	29.82 (0.06) a	7.36 (0.04) a	1775 (9) a
AOV	2.11^{ns}	198.1***	7.19*	0.12^{ns}	4.55^{ns}	11.9*

Each value is the treatment mean with standard error in parentheses for 24 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as *F* value followed by *P* category: ^{ns}non-significant; *significant at $P < 0.05$; **significant at $P < 0.01$; ***significant at $P < 0.001$. *AOV* is analysis of variance. 3 month post treatment period for the early season drought treatment was between February and April, for the late season drought treatment between June and August and the summer drought treatment between May and July.

At a monthly level, treatment level correlations between growth rate and wood properties were significant ($P < 0.001$). Density and *E* exhibited a moderately negative correlation with growth rate ($r = -0.64$ and -0.65, respectively), whilst MFA exhibited a moderately positive correlation with growth rate ($r = 0.69$, Figure 4.4). No significant relationships ($P > 0.05$) at the tree or treatment level were found between ring width, acting as a surrogate for growth rate, and wood properties.

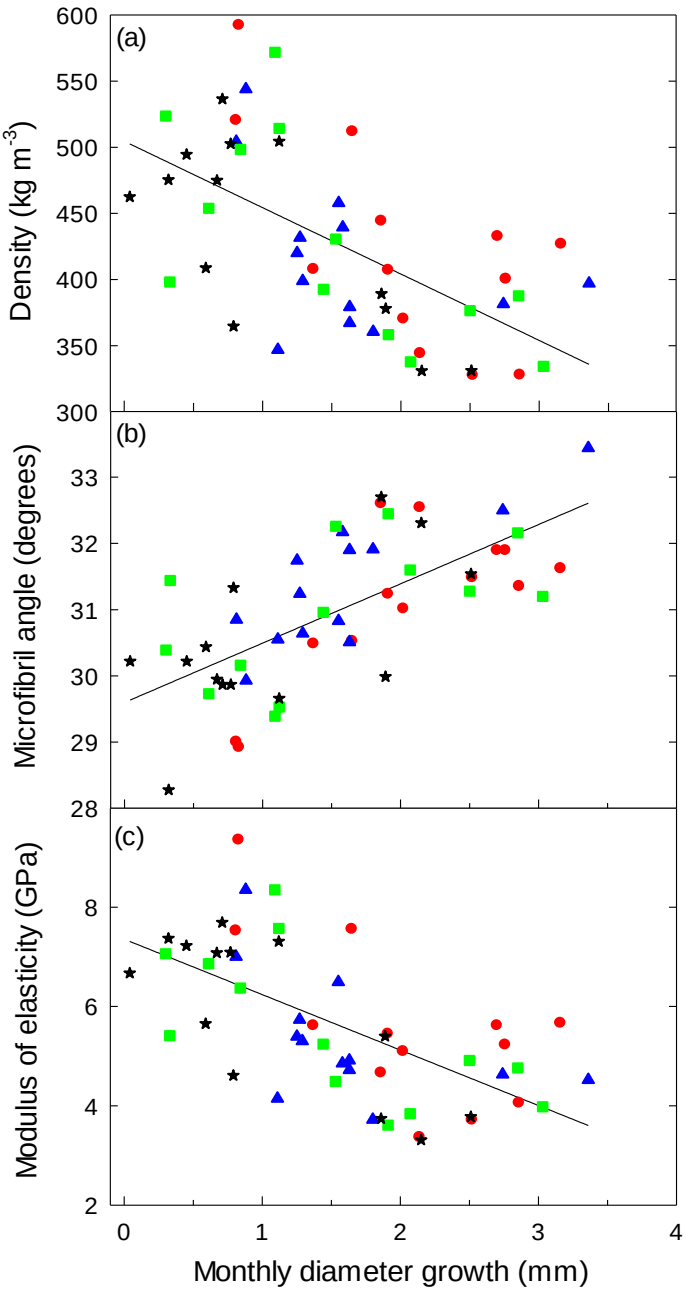


Figure 4.4. Treatment level relationship between monthly diameter growth and monthly average values of (a) density, (b) microfibril angle and (c) modulus of elasticity for the well watered (red circle), early season cyclical drought (blue triangle), late season cyclical drought (green square) and summer drought (black star) treatments. A line of best fit for all treatments is drawn for each relationship.

There were no statistically significant differences in measures of average density, MFA and *E* between the two pith to bark radial samples representing opposite sides of the tree across all trees in the experiment. Across all trees, nine trees exhibited some moderate tangential variation with differences in average *E* between the two sides of the tree greater than 0.8 GPa (Figure 4.5). Nevertheless, the average tangential variation in *E* of 0.42 GPa was dwarfed by the average radial variation in *E* of 8.5 GPa.

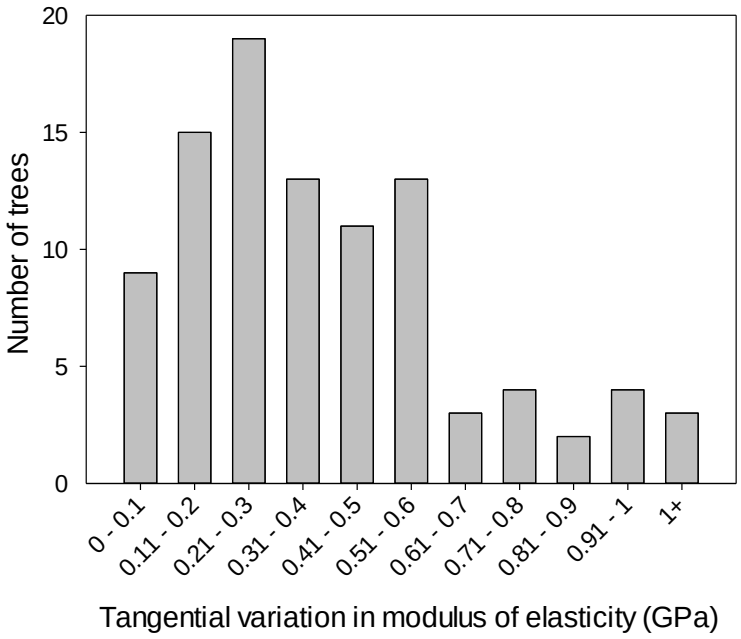


Figure 4.5. Absolute differences in tangential variation for average modulus of elasticity between the two pith to bark radial samples.

DISCUSSION

The literature on wood property variation is often confused and contradictory, especially when addressing its relationship with growth rate (Downes and Raymond, 1997). The difference between seasonal and annual results in this experiment adds to the conflicting information from numerous recent studies regarding the relationship between growth and wood properties in young *P. radiata* (Watt *et al*., 2005; Mason, 2006; Lasserre *et al*., 2008; Xue *et al*., 2013; Nanayakkara *et al*., 2014). Growth rate can, and has been defined in terms of radial increment per day, week, month, year or years. However, these are very different measures of time, both qualitatively and quantitatively (Downes *et al*., 2002). Most studies are based on annual or greater timescales. The findings of this experiment are consistent with other sub-annual studies examining the direct impact of water availability on wood properties which have demonstrated that density was lower and MFA was

higher in faster growing compared to slower growing trees (Downes *et al*., 2004; Watt *et al*., 2005; Drew *et al*., 2009; Drew *et al*., 2011). Seasonal water deficits however, had no impact on annual average values of density, MFA and *E* in juvenile *P. radiata* in this experiment.

The lack of variation in annual values of density, MFA and *E* was of particular interest given the implications for forest growers. Variations in wood properties during imposition of water deficits were completely offset by post treatment values of wood properties for density, MFA and *E*. This result may suggest that whilst water deficits are important at the sub-annual level, they are not at the annual level. Previous work by Cown *et al*. (1991), Watt *et al*. (2006) and Watt and Zoric (2010) across nationwide gradients in environment, covering the range in which *P. radiata* is grown, have shown that temperature is considerably more important in determining wood properties than measures of water availability. However, a woody weed control study at a single site where water deficits were indirectly applied, found that trees with weeds and greater levels of water stress had significantly ($P < 0.05$) higher values of density and *E*, and lower values of MFA, compared with the trees without weeds and lower levels of water stress (Watt *et al*., 2005) over multiple years. This study only indirectly applied water deficits and trees were subject to other influences such as wind induced tree sway, which has previously been shown to induce increases in MFA and reductions in *E* (Nicholls, 1982; Telewski and Jaffe, 1986; Pruyn *et al*., 2000). In the context of this experiment, the lack of annual variation in density, MFA and *E* between treatments is a positive finding and of interest to forest growers as these results suggest that achieving fast growth in trees is not detrimental to internal wood properties in juvenile *P. radiata*.

The impact of water deficits on stem diameter growth was more pronounced for the late season drought treatment compared with the early season drought treatment. As a result, measures of density and *E* were greater and MFA lower for the late season drought treatment than for the early season drought treatment, when compared with the well watered treatment during periods of imposed water deficits. The effect of water deficits on the summer drought treatment was to dampen variation in intra-annual values of density, cell wall thickness and *E*. It is possible that this would result in lower differential shrinkage within the annual ring.

Latewood percentage was significantly different between the summer drought treatment and the others, however, this difference did not translate into increased annual average values of density. This can be attributed to the fact that the summer drought treatment had the lowest average earlywood values of density and maximum values of latewood density were less than the other treatments during winter. The water deficit treatments induced latewood formation at very different times of the year with the early season drought delaying formation by approximately two months while late season drought induced formation about 10 days earlier compared with the well

watered treatment. Summer drought induced latewood formation in late December, a couple of months earlier than the well watered treatment which is very similar to observations made in *P. resinosa* by Zahner *et al*. (1964). This experiment in conjunction with others demonstrates that latewood initiation can be promoted by increased evaporative demand or reduced water availability (Whitmore and Zahner, 1966; Brix, 1972; Cregg *et al*., 1988). However, latewood formation ceased on about the same date for all treatments irrespective of previous water status demonstrating an overriding phenological response to an environmental stimulus.

In addition to significant effects of water deficits on wood properties, resumption of normal water availability also resulted in large differences in wood properties compared to the well watered treatment. The improved water status of the seasonal drought trees post treatment and subsequent increases in growth rates, resulted in declines in density, cell wall thickness and *E*, and increases in MFA relative to the well watered treatment. Water deficits had little effect on cell wall thickness during imposition of treatments, supporting the work of Sheriff and Whitehead (1984), however post treatment, significant differences were observed in cell wall thickness between the well watered and water deficit treatments.

Changes in wood properties throughout the year, independent of water deficit treatments, followed expected patterns of formation. This was particularly evident for density, cell wall thickness and *E*, whereby lower values were observed early in the growing season and higher values were reached in winter, similar to patterns observed by Watt *et al*. (2005). Changes in MFA were less pronounced, however a general decline in values for all treatments during the course of the year was observed. Typically in softwoods, MFA varies markedly between wood formed in spring and wood formed in summer and autumn (McMillin, 1973; Downes and Drew, 2008), however, average MFA values changed by only approximately two degrees between seasons for all treatments in this experiment. This range in variation is considerably less than that observed by McMillin (1973) in *P. taeda* and Watt *et al*. (2005) in juvenile *P. radiata*. The substantial increase in *E* over the course of the year for all treatments demonstrates the significant differences in earlywood and latewood *E* within the annual ring, with *E* at least doubling during the course of the year for all treatments.

Tangential variation in density, MFA and *E* between the two pith to bark radial samples from each tree was not significant, however, approximately 10% of the population exhibited tangential differences in *E* greater than 0.8 GPa, with one tree displaying a 3.5 GPa difference in *E*. Given that average tangential variation across the sampled population in this experiment was considerably smaller than radial variation in *E*, further wood properties research may benefit from significant cost savings as only samples from one side of tree may be required for high resolution testing and examination. Such consistency between sides of the tree would undoubtedly be of reassurance to

wood processors and have important implications for forest managers attempting to achieve a uniform crop.

CONCLUSIONS

In conclusion, this experiment highlights the effects that the timing and duration of water deficits has on regulating wood properties of juvenile *P. radiata*. Decreased productivity resulting from restricted water availability is unfavourable but results from this experiment show that at the intra-annual level, this is associated with an increase in wood density and *E*, and an decrease in MFA. Water deficits significantly ($P < 0.05$) increased density and *E* compared with the well watered treatment. Seasonal differences were most evident for the late season drought treatment which increased density by 10%, *E* by 18% and decreased MFA by 5% compared with the well watered trees. At the annual level, differences in productivity had no impact on values of density, MFA and *E* as post treatment values of wood properties for the water deficit treatments completely offset earlier values of wood properties for density, MFA and *E*. This suggests that at the ring level, the benefits of increased productivity will not be offset by lower timber quality in juvenile *P. radiata*.

CHAPTER FIVE

ACOUSTIC VELOCITY AND LONGITUDINAL SHRINKAGE OF JUVENILE *PINUS RADIATA* IN RESPONSE TO WATER DEFICITS

SUMMARY

The influence of four levels of water supply on acoustic velocity and longitudinal shrinkage in stems of two-year-old *Pinus radiata* D. Don were examined in a controlled environment. Acoustic velocity of wood is related to important wood quality properties such as the microfibril angle of the S2 layer in the cell wall, stiffness, and shrinkage propensity. Well watered trees exhibited higher velocity and also greater longitudinal shrinkage which were both significantly different ($P < 0.05$) from the water deficit treatments. Within treatment variation for acoustic velocity and longitudinal shrinkage was greatest for the well watered treatment. Weak to non-existent relationships were observed between longitudinal shrinkage and other wood properties.

INTRODUCTION

Advances in tree breeding and changes in silvicultural practice over the last few decades have greatly enhanced growth rates of plantation-grown conifers. These growth gains have led to shorter rotations and an increased proportion of poor quality corewood (Cown, 1992), defined as that wood closest to the pith. Physical and mechanical properties of corewood differ from those of mature wood (DeBell *et al.*, 2002) and it is widely accepted that coniferous trees grown in plantation conditions have corewood with physical features that are inferior in comparison to those found further from the pith (Butterfield, 2003). Corewood is generally characterised by low density, thin cell walls, short fibres with small lumens, high grain angle, and high microfibril angle (MFA), with the result that it has low strength and modulus of elasticity (*E*) and poor dimensional stability compared to mature wood (Macdonald and Hubert, 2002).

Wood products are distinguished by their mechanical performance in structural applications. Wood stiffness, measured as modulus of elasticity (*E*), and stability are important properties of the widely grown plantation softwood *Pinus radiata* D. Don for end-user applications. High values of *E* and good stability are essential for solid timber applications and structural wood based composites that affect customer perceptions of value in both structural and appearance products (Cown *et al*., 1999). Consequently, the end-use of wood is strongly related to *E* and stability. When compared to other internationally traded structural lumber species, plantation-grown *Pinus radiata* has relatively poor *E* (Walford, 1991) and dimensional stability.

Modulus of elasticity measures the resistance of wood to deformation under load and is used as a threshold criterion in machine stress grading of structural timber (Walker and Nakada, 1999). It is also a key property for determining quality of laminated veneer lumber. *E* is often considered more important than strength (modulus of rupture) for predicting wood quality, because *P. radiata* boards rarely break in normal use; much more frequently a load results in excessive deflection (Walford, 1985). Acoustic velocity is an effective surrogate measure of *E*, while also reflecting and capturing other wood characteristics such as fibre length, MFA and even the chemical composition of cell walls (Albert *et al*., 2002; Huang *et al*., 2003).

Dimensional stability is a major concern during processing and utilisation when it is subjected to fluctuating temperature and relative humidity (Pang and Herritsch, 2005). Stability is ultimately a measure of differential shrinkage and influences the incidence of bow, crook and twist. Good stability requires low values of differential shrinkage. Shrinkage is the reduction in dimensions of timber during drying due to the movement of moisture out of cell walls of the wood. The amount of shrinkage is generally proportional to the amount of cell wall material and the amount of

moisture removed, and thus shrinkage increases in proportion to wood density. Wood shrinkage occurs by the removal of water bound to the cellulose and hemicellulose molecules. The region consisting of non-crystalline cellulose and hemicellulose shrinks in the direction that is orthogonal to the crystalline cellulose microfibrils. Therefore, longitudinal shrinkage increases and transverse shrinkage decreases with an increase in the microfibril angle (Megraw *et al*., 1998; Yamashita *et al*., 2009) but in a curvilinear manner (Walker and Butterfield, 1996; Megraw *et al*., 1998). The higher MFA found in the inner corewood means that longitudinal shrinkage in *P. radiata* is mainly a problem in the first three growth rings (Harris and Cown, 1991) and is worse in the butt log of the tree than higher up (Megraw *et al*., 1998; Wang *et al*., 2008; Sialumba, 2011). It is particularly responsible for crook (Walker and Butterfield, 1996; Johansson, 2003).

Widespread adoption of machine stress grading of timber has highlighted both the substandard nature of the New Zealand *P. radiata* resource and the financial gains that would be possible if *E* in corewood could be increased sufficiently to reach thresholds for structural grades (Walker and Nakada, 1999). Low values of *E* and high longitudinal shrinkage present in the inner corewood of *P. radiata* limits its utilisation, however, current breeding programs are attempting to identify clones with high *E* (Jayawickrama, 2001; Kennedy *et al*., 2014), which would also result in decreased anisotropic shrinkage. If suitable clones are found that produce wood that meets the minimum requirements for New Zealand structural grades, significant gains in value will be realised from improved sawmilling recovery rates (Walker and Butterfield, 1996; Dickson and Walker, 1997; Watt *et al*., 2010). However, such improvements may be diminished or completely undermined if the effects of site, silviculture and environment on important harvest age properties are not clearly understood.

Recent research shows that in plantation-grown *P. radiata*, corewood properties can be manipulated by management practices. Significant improvements in corewood properties have been shown through increasing initial stand density (Lasserre *et al*., 2009), reducing the level of woody weed control (Watt *et al*., 2005), using clones with improved wood properties (Lindström *et al*., 2004) and selection of site (Watt *et al*., 2006). However, despite an improved understanding of how environment influences corewood properties (Watt *et al*., 2009), there is little information on velocity and shrinkage of *P. radiata* corewood in response to water deficits.

Pinus radiata is likely to be subjected to increasingly erratic seasonal rainfall, more frequent and severe drought, and increased evaporative demand brought about by higher temperatures under predicted climate change in decades to come (Kirschbaum and Fischlin, 1996; Kirschbaum *et al*., 2012). At present, soil water deficits are common across many eastern regions of New Zealand during summer (Palmer *et al*., 2009) and many of these areas are predicted to receive less rainfall in

the future[6] with an increased risk of severe drought (Mullan *et al*., 2005). Forest managers can also influence soil water availability through choices in site, stand density and level of weed control (Nambiar, 1990; Stogsdili *et al*., 1992; Yunusa *et al*., 1995*a*; Watt *et al*., 2003; Watt *et al*., 2006).

A thorough understanding of the *P. radiata* corewood zone and its response to a range of environmental conditions is important for forest managers and solid wood processors in order to improve wood properties and the grade out turn in this zone of the tree. The primary objective of this experiment was to quantify the impact that the timing and duration of water deficits has on corewood acoustic velocity and longitudinal shrinkage in juvenile *P. radiata* over the course of one growing season.

METHODS

Experimental design and treatments

The experiment was undertaken in a polyhouse which provided growing conditions where water supply to *Pinus radiata* D. Don clonal stock could be controlled. The clone used was a high wood density, high acoustic velocity clone (Clone 35, Forest Genetics CellFor Ltd) produced using somatic embryogenesis. The nursery raised trees were planted in the polyhouse for a two year period and the experimental treatments were applied during the second year. The trees were planted in a free draining loamy sand soil in 200 litre pots to reduce any potential for water logging and root binding. Soil texture and particle size were determined prior to experiment commencement using a hydrometer for smaller particles and sieve for larger particles. Particles were separated into <0.002, 0.002-0.06, 0.06-0.2, 0.2-0.6 and 0.6-2.0 mm size classes. Medium and coarse sand compromised 76% of the soil.

The clonal tree material grown in the polyhouse was subjected to four water deficit treatments laid out in six blocks of five rows for one growing season (August 2009 to September 2010). Within each block of five rows, all four water deficit treatments were present plus one buffer row. Each treatment, which occupied one row, consisted of four trees. The treatments within each block were assigned randomly. The buffer row within each block was placed systematically on the next row to the north of rows subject to the summer drought treatment so that shading of the drought trees and thus any confounding influences were minimised. Trees were spaced 1.4 m apart

[6] http://www.mfe.govt.nz/publications/climate/climate-change-effect-impacts-assessments-may08/page4.html

so that there was no physical interaction between their crowns. Twenty four trees per treatment were present giving a total of 96 trees.

Four water deficit treatments were applied to the experimental trees. Irrigation was applied using drip sprinklers until pre-specified predawn needle water potentials were reached. The first treatment was 'well watered' with applications of water applied weekly between October and May (spring to autumn), and fortnightly between June and September (winter). The second treatment simulated 'extended summer drought' whereby water was withheld between November and April to allow drying of the soil profile. The drought was interrupted by the small, single application of water on March 1 to prevent tree mortality. Although predawn needle water potentials were not particularly low at this time, the trees had started showing large physical changes to continued drought. During the remainder of the year, water was applied at the same times and rates as the well watered treatment. The third and fourth treatments simulated 'early season cyclical drought' and 'late season cyclical drought', respectively. The early season cyclical drought treatment was applied between October and January (spring/early summer), while the late season cyclical drought treatment was applied between February and May (late summer/autumn). These treatments were timed to coincide with periods of expected early- and latewood development. Trees in these treatments were subjected to a series of drying cycles interrupted by water replenishment when needle water potential had fallen to approximately -0.5 MPa or lower. This water potential was sufficient to generate a depressed growth response as previously observed in non-experimental trees. The early season drought trees were re-watered on November 7, December 1, December 26 and January 16, while the late season drought trees were re-watered on February 20, March 13 and April 19. When not subjected to the cyclical watering treatments between the stated months, water for both treatments was applied at the same times and rates as that for the well watered treatment. The length of time between watering for the cyclical drought treatments depended upon the time of year and air temperature. The time between watering varied from three to seven weeks during the October to May period.

Environmental variables within the polyhouse were measured throughout the duration of the experiment using sensors connected to a data logger (HOBO weather station, ONSET Computer Corporation, Bourne, MA, USA). Air temperature fluctuated between -1 and 35 °C with a mean and standard deviation of 14.3 ± 5.9 °C. Monthly measures of soil temperature closely corresponded to mean air temperature throughout the course of the experiment (data not shown). Vapour pressure deficit fluctuated between 0.01 and 3.89 kPa with a mean and standard deviation of 0.41 ± 0.53 kPa, while the maximum value of photosynthetically active irradiance was 1023 µmol m^{-2} s^{-1} with a mean and standard deviation of 134 ± 234 µmol m^{-2} s^{-1}.

Predawn needle water potential and measurements of tree growth

Measurements of predawn needle water potential (ψ_e) were made throughout the experiment using a pressure chamber. Three trees per treatment were sampled on each occasion. The timing of sampling depended on the season with weekly sampling occurring in the summer months and fortnightly sampling occurring in the winter months. From each tree, three fascicles were sampled from the youngest well developed foliage. The fascicles were collected before dawn and stored in test tubes on ice until ψ_e was measured, always within one hour of collection. Following Myers (1988), water stress integral (S_ψ) was determined as the cumulative integral of predawn needle water potential over the period which water deficits were imposed (1 October to 31 May). Values of S_ψ are expressed as an absolute sum, so that larger values of S_ψ represent greater accumulated water deficits.

Measurements of tree growth were taken during the second year that the trees were planted in the polyhouse. Monthly measurements of total tree height and diameter (at a tree height of 0.5 m) were made. Measures of diameter were made on a single axis for all trees using electronic calipers. Stem slenderness was calculated as the ratio of tree height to ground-line diameter. Basal area was calculated using Husch *et al*. (2003).

Acoustic velocity

At the conclusion of the experiment all 96 trees were destructively sampled. Whole disk samples 40 mm thick were cut at ca. 0.5 m tree height, immediately above the disk used for Silviscan analysis (Chapter 4). The disk samples were conditioned to 12% moisture content (MC) at 65% relative humidity and 20 ^{o}C. Using the disk samples, all 96 trees were tested using an ultrasonic velocity (USV) scanner, TreeDisk. This scanner measures ultrasonic velocity produced from pulse transducers calibrated for disk thickness prior to scanning and has been shown to be strongly correlated (r^2 = 0.89, P < 0.001) with ring level estimates of microfibril angle derived from x-ray diffraction (Sialumba, 2011). The USV scanner is a single stress wave time of flight tool. The USV scanner is able to provide 5 mm resolution incremental measures of velocity using a programmed grid scanning map which is digitally overlaid and image analysis to identify scanning boundaries and the pith. The untreated first year wood was excluded from the analysed dataset.

Longitudinal shrinkage

Measures of longitudinal shrinkage were made on all 96 trees. Bolt samples 100 mm in length were taken from each tree at a tree height of ca. 0.65 m. The samples at this height only contained a small amount of non-treated first year xylem and primarily consisted of treated second year xylem. The bolts were split into two along the pith in the east/west orientation to give two samples per tree (total of 192 samples). The samples were machined using a band saw to produce a sample roughly 15 x 20 x 100 mm in size.

Two spherically headed pins were inserted on the opposite end faces of each sample. The pin heads provided the reference points for repeated length measurements. Each sample was located by its pins in a jig (Figure 5.1) and a micrometer reading was recorded. The measurements were highly repeatable, which is essential for accurate longitudinal shrinkage measurements. Measures of green length were taken. The samples were then conditioned to 12% moisture content at 65% relative humidity and 20 ^{o}C. Once the samples had reached equilibrium, they were remeasured for length using the jig. A few samples exhibited a small amount of bow on drying and for these, the dried sample length was adjusted for bow using the Pythagorean theorem:

$$\textit{Corrected length} = \sqrt{(Dry\ length)^2 + (Bow)^2} \quad (1)$$

Longitudinal shrinkage was calculated using the following formula:

$$\textit{Longitudinal shrinkage (\%)} = \textit{Green length} - \frac{Corrected\ length}{Green\ length} \times 100 \quad (2)$$

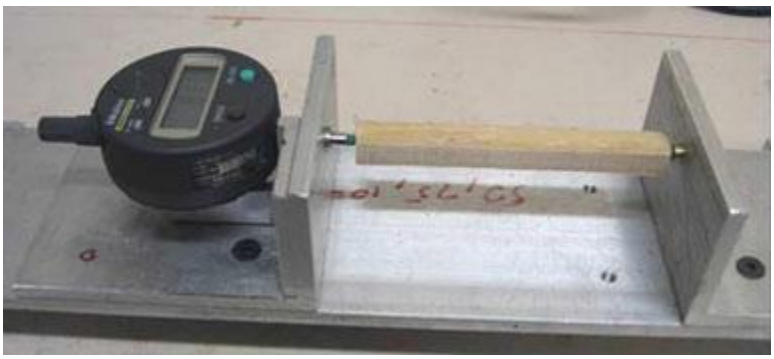


Figure 5.1. Device for measuring longitudinal dimensions in short samples.

Following this measurement the pins were removed and the samples were tested for acoustic velocity using the following acoustic resonance technique. A stress wave was generated at

one end of the sample by tapping the cut face of the sample with a small ball hammer while a microphone at the other end captured the vibrations caused by the stress wave. The signals were then analysed using a digital Fourier transform which allowed the fundamental resonance frequency of the longitudinal vibrations to be determined. The acoustic resonance technique is an accurate and repeatable way of determining sound speed. Using material from four-year-old *P. radiata* clones, Lindström *et al*. (2002) found that correspondence between *E* measured using the resonance technique and traditional static bending was very strong (r^2 = 0.98) and relatively unbiased (y = 1.04x). The resonance measurement of velocity is a near perfect spatial average of the sample, both in sample length and cross-sectional area. Acoustic velocity (V) was determined from the fundamental frequency (f) and sample length (l) using the following formula:

$$V = 2l f \tag{3}$$

Air-dried density was determined from sample weight/sample volume. Sample volume was measured under water using Archimedes' principle. In order to avoid any air bubble formation on wood surfaces during immersion in water, samples were wiped on all surfaces using a damp cloth just prior to immersion and measurement conducted quickly to avoid any water absorption. Modulus of elasticity (E) was derived from V and air-dried density (p) using the following formula:

$$E = pV^2 \tag{4}$$

Tree level measures of microfibril angle (MFA) and modulus of elasticity (referred to as SS*E* in this chapter) estimated using Silviscan are included in this chapter. Refer to Chapter 4 for Silviscan methodology.

STATISTICAL ANALYSIS

All analyses were carried out using R software (R Core Team, 2013). There were no statistically significant treatment differences in any of the measured variables at the start of the experiment. The effect of water deficits on tree growth, acoustic velocity and longitudinal shrinkage were examined using a mixed effects model that included treatment as a fixed effect with block as a random effect as follows:

$$Y_{ij} = X_i \beta + Z_j b_j + e_{ij} \tag{5}$$

where Y_{ij} is the observation vector of the response variable, X is the fixed effect of treatment, Z is the random effect of block and e is the error term. The subscripts $_i$ and $_j$, represent treatment and block, respectively. β is the vector of fixed effect coefficients, and b is the vector of random effect coefficients. The random effect, b_j, and error, e_{ij}, are independent and normally distributed with mean zero and variances σ_b^2 and σ_e^2, respectively.

Linear models were developed to examine the relationship between ultrasonic velocity and silviscan measures of microfibril angle and modulus of elasticity using the following form:

$$y = a + bx \quad (6)$$

where y is either microfibril angle or modulus of elasticity, a is the intercept, b is the slope and x is ultrasonic velocity.

Spearman's correlation analysis was performed to assess the relationship between ring width and within tree variation in velocity, and between longitudinal shrinkage and wood properties using tree level data. Tukey's HSD test was used, where applicable, to distinguish between treatment mean values. Differences were considered significant at $P < 0.05$.

RESULTS

Predawn needle water potential

The implementation of the early season cyclical drought, late season cyclical drought and summer drought treatments had a pronounced effect on ψ_e and induced consistently more negative values of ψ_e than for the well watered trees (Figure 5.2). The early season and late season cyclical drought treatments resulted in large fluctuations of ψ_e. In both cases, application of water decreased ψ_e immediately and usually, dramatically. The summer drought treatment induced consistently more negative values of ψ_e during the period between mid-December and April. Values of S_ψ were 41.4, 66.8, 55.2 and 97.6 MPa-days for the well watered, early season drought, late season drought and summer drought treatments, respectively.

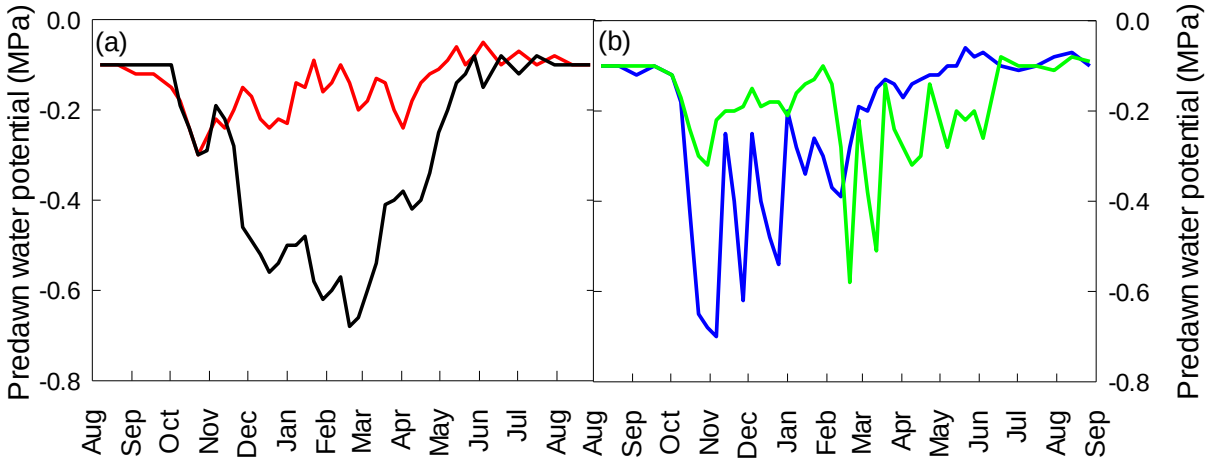


Figure 5.2. Seasonal changes in predawn needle water potential for (a) the well watered (red line) and summer drought (black line) treatments, and (b) early season cyclical drought (blue line) and late season cyclical drought (green line) treatments.

Tree characteristics

Tree height, diameter at 0.5 m and basal area were all significantly influenced by treatment ($P < 0.001$). Stem slenderness was also significantly influenced by treatment ($P = 0.012$), but only due to the variation in slenderness between the well watered and early season drought treatments. Basal area was the most sensitive indicator of water deficits (Table 5.1). With the imposition of water deficits, marked divergence in monthly diameter (Figure 5.3) and height growth became immediately apparent. Decreased growth for all water deficit treatments coincided with more negative predawn needle water potential values. There were no significant differences ($P > 0.05$) in height, diameter and basal area between early and late season drought trees at the end of the experiment (Table 5.1) although S_{ψ} was larger in the early season drought trees than in the late season drought trees. Compared to the well watered treatment, the summer drought treatment reduced height, diameter and basal area by 24.7%, 33.1% and 52.3%, respectively.

During the period of imposed water deficits for the early season drought between October and January, trees increased in diameter by 5.36 mm compared to 10.60 mm for the late season drought trees and 10.17 mm for the well watered trees. When water deficits were imposed for the late season drought trees between February and May, diameter increased by 2.37 mm compared to 8.90 mm for the early season drought and 9.41 mm for the well treatment trees. Figure 5.3 demonstrates that the reduction in stem diameter growth was higher for the late season drought treatment than the early season drought treatment when compared with the well watered trees,

suggesting that late season drought is more detrimental than early season drought to tree growth. The summer drought trees increased in diameter by 2.85 mm compared with 15.41 mm for the well watered trees.

Table 5.1. Treatment variation in tree dimensions at the end of the experiment.

Treatment	Tree height (m)	Diameter (mm)	Basal area (mm^2)	Slenderness ($m\ m^{-1}$)
Well watered	3.54 (0.55) a	38.26 (0.80) a	1590 (76) a	79.6 (1.2) a
Early season drought	3.22 (0.53) b	32.37 (0.59) b	1086 (35) b	87.0 (1.0) b
Late season drought	2.99 (0.59) b	30.61 (0.58) b	1015 (38) b	83.8 (1.3) ab
Summer drought	2.61 (0.42) c	25.59 (0.42) c	759 (21) c	84.1 (1.2) ab
Analysis of variance				
Treatment	37.3***	44.2***	34.2***	5.12*

Each value is the treatment mean with standard error in parentheses for 24 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$. Significance of treatment effects is shown as F value followed by P category: *significant at $P < 0.05$; ***significant at $P < 0.001$.

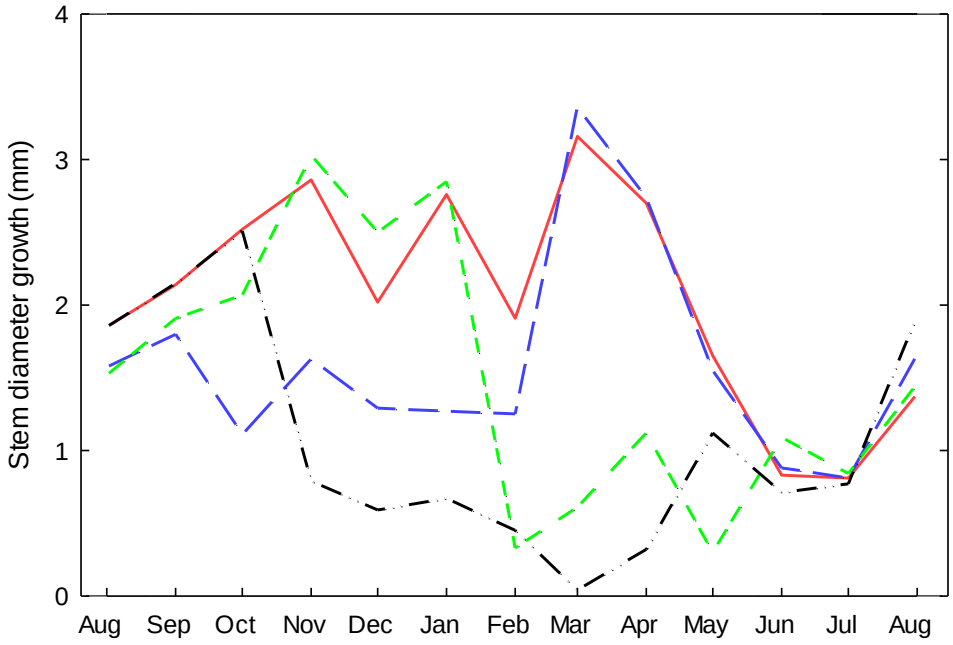


Figure 5.3. Average monthly incremental stem diameter growth for the well watered (solid red line), early season cyclical drought (long blue dash), late season cyclical drought (short red dash) and summer drought (black dash-dot-dot) treatments.

Ultrasonic velocity

Significant treatment differences (P = 0.028) existed for measures of USV (Table 5.2), driven by the higher average velocity for the well watered treatment. The average within tree variation in velocity was greatest for the well watered treatment while the seasonal drought treatments were considerably lower and similar to each other. There was a significant positive relationship between ring width and within tree variation in velocity (r = 0.38, P < 0.001).

Table 5.2. Treatment effects on ultrasonic velocity.

			Within tree variation in velocity			
Treatment	Mean (m s^{-1})	SE (m s^{-1})	Min (m s^{-1})	Max (m s^{-1})	Avg. (m s^{-1})	Count
Well watered	4066 a	8.36	377	1334	698	867
Early season drought	3934 ab	6.78	271	887	485	585
Late season drought	3950 ab	7.70	281	772	444	514
Summer drought	3928 b	12.1	194	766	494	376

Treatment means and standard error are presented for 24 trees. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at P < 0.05. Min and Max are the minimum and maximum values of within tree variation for that treatment. Avg. is the average within tree variation in velocity across all trees within treatment. Count refers to the number of sample points taken by the USV scanner for all trees in that treatment.

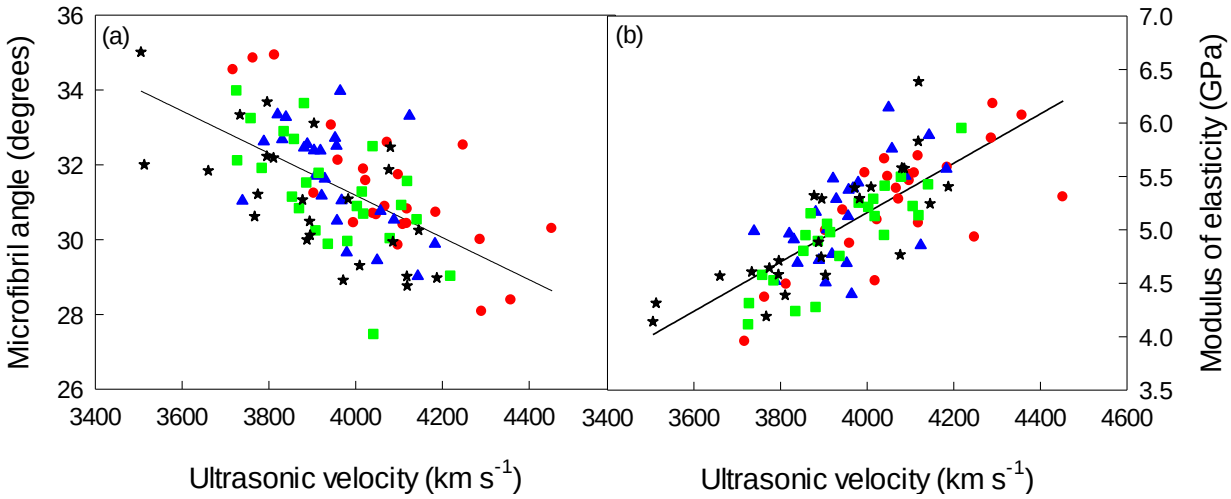


Figure 5.4. Relationships between ultrasonic velocity and (a) microfibril angle, and (b) modulus of elasticity from Silviscan for the well watered (red circle), early season cyclical drought (blue triangle), late season cyclical drought (green square) and summer drought (black star) treatments. A line of best fit for all treatments is drawn for each relationship.

Measures of ring level USV were found to be significantly related ($P < 0.001$) to ring level Silviscan measures of MFA and modulus of elasticity (SS*E*). The relationship between USV and MFA (Figure 5.4, *above*) was found to have a moderately negative correlation ($r^2 = 0.36$), while measures of USV and SS*E* were strongly positively correlated ($r^2 = 0.61$). A large range existed between the fastest (4450 m s^{-1}) and slowest (3512 m s^{-1}) average velocity trees within the experiment (Figure 5.5).

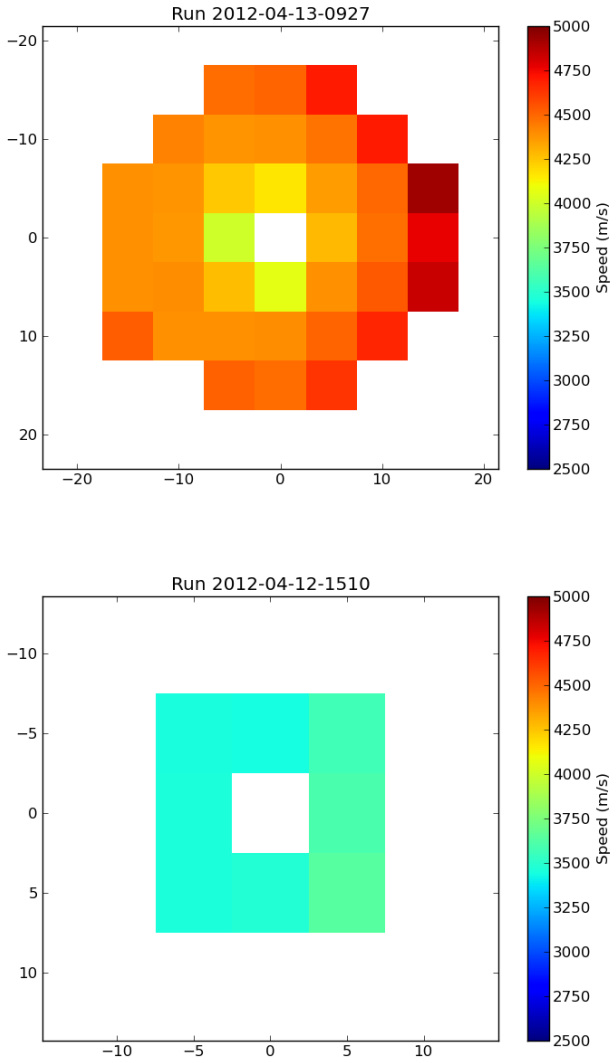


Figure 5.5. Ultrasonic velocity profiles for the fastest (top) and slowest (bottom) velocity trees.

Longitudinal shrinkage

Longitudinal shrinkage significantly differed (Table 5.3) between the well watered treatment and the seasonal drought treatments ($P < 0.05$). The range in absolute values of shrinkage for all 192 samples was small (max = 1.05%) with 184 of the 192 samples exhibiting values of longitudinal shrinkage between 0.4% and 0.9%. The higher longitudinal shrinkage exhibited by the well watered treatment

was driven by higher values of shrinkage for the east side samples of 14 trees (Figure 5.6). The corresponding sample from the west side of each of these trees was on par with shrinkage values for the seasonal drought treatments. This difference can most likely be explained by the fact that *E* for the west side samples of these trees was on average 0.42 GPa stiffer than the east side samples. For all remaining trees in the experiment, this difference between sides was on average only 0.09 GPa. The reasons for this anomaly are not clear.

Table 5.3. Treatment effects on longitudinal shrinkage.

Treatment	Mean (%)	SE (%)	CV (%)	Min (%)	Max (%)
Well watered	0.65 a	0.02	12.3	0.28	1.03
Early season drought	0.59 b	0.02	15.3	0.15	0.95
Late season drought	0.60 b	0.02	13.3	0.28	1.05
Summer drought	0.58 b	0.02	12.1	0.22	0.80

The mean, standard error and coefficient of variation are treatment values for 24 trees. Min and Max are at the sample level showing the range within treatment for 48 samples. The Tukey HSD test was performed between treatments. Means followed by the same letter are not significantly different at $P < 0.05$.

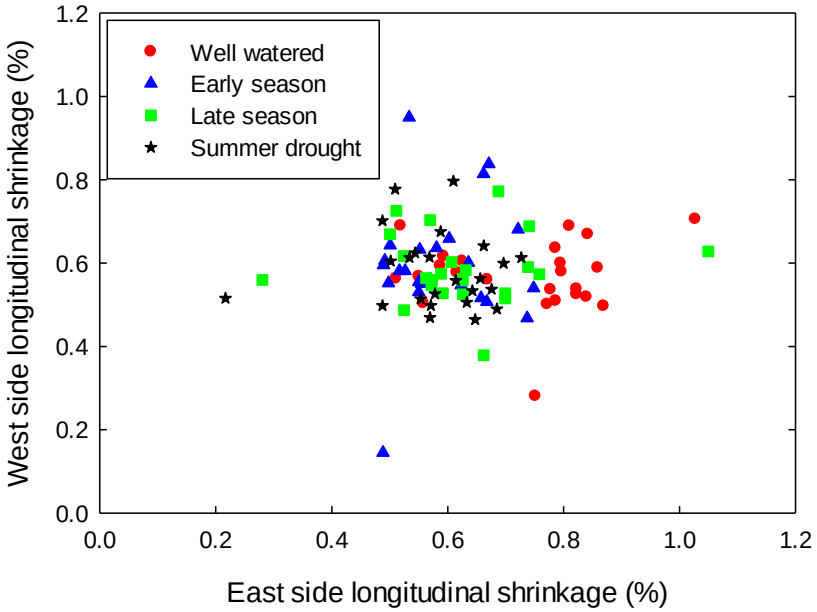


Figure 5.6. Relationship between measures of longitudinal shrinkage from the two samples obtained from the east and west sides of each tree.

Tree level correlations between longitudinal shrinkage and MFA, air-dried density, *E* and ultrasonic velocity were examined. There was a very weak positive but non-significant correlation ($r = 0.17$, $P = 0.093$) between shrinkage and MFA. No correlation existed between shrinkage and air-

dried density and between shrinkage and ultrasonic velocity (Figure 5.7). The statistically significant negative correlation between shrinkage and *E* was stronger (r = -0.25, P = 0.014).

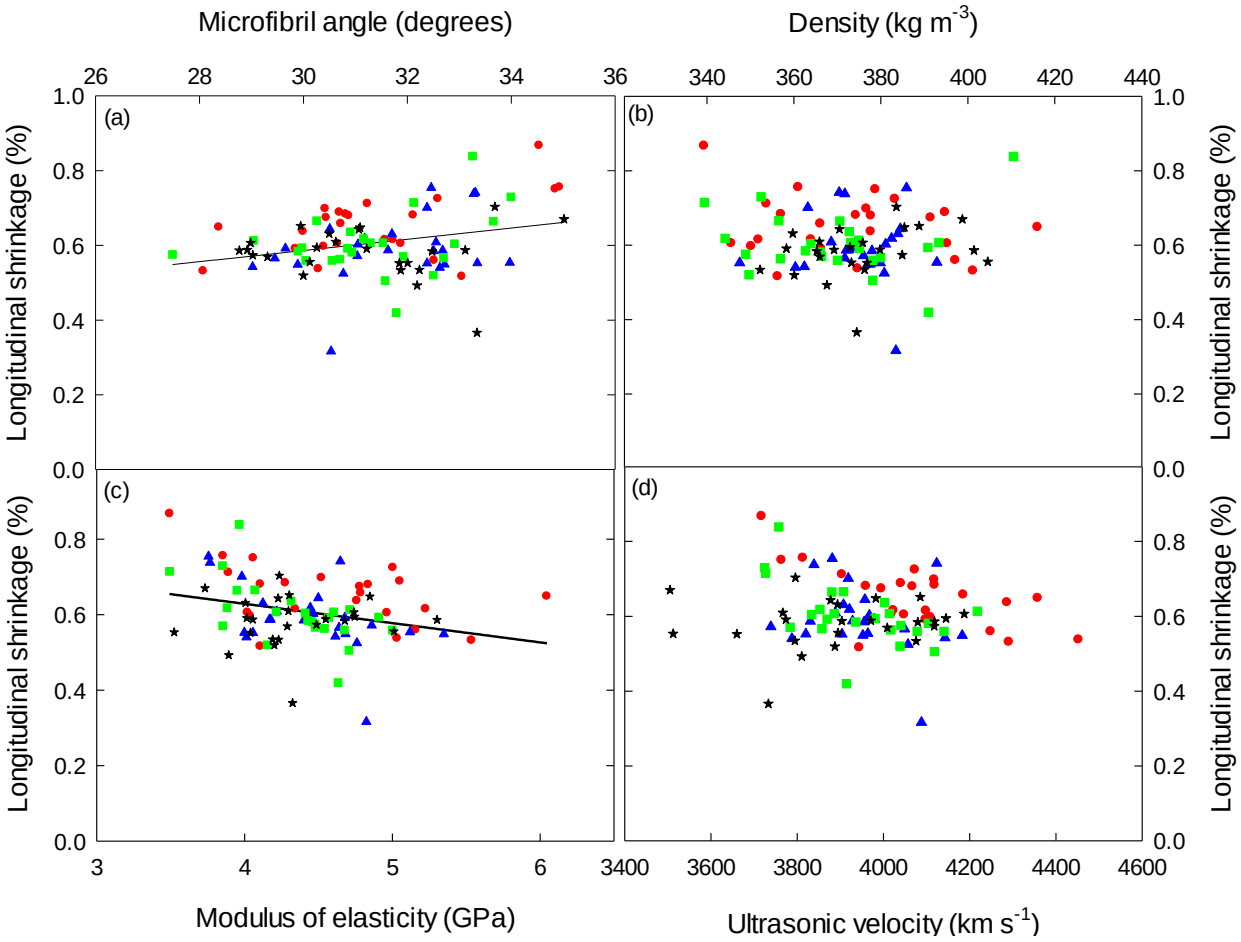


Figure 5.7. Relationships between longitudinal shrinkage and (a) microfibril angle derived from Silviscan samples, (b) air-dried density of the shrinkage samples, (c) modulus of elasticity calculated from velocity and density of the shrinkage samples at 12% MC, and (d) ultrasonic velocity, for the well watered (red circle), early season cyclical drought (blue triangle), late season cyclical drought (green square) and summer drought (black star) treatments. A line of best fit for all treatments is drawn for graphs and (a) and (c).

DISCUSSION

Mixed results were observed in this experiment with the well watered treatment having higher velocities indicating stiffer wood, but yet having higher average shrinkage. However, treatment level differences in absolute values were relatively small, even if they were statistically different. The large differences in tree size and growth rate between the treatments did not translate into large differences in wood properties. The small differences in acoustic velocity between treatments suggest that differences in their intrinsic microfibril angle may be very small, as velocity is considered

to be a surrogate measure of MFA (Huang *et al*., 2003; Huang and Lambeth, 2007). This agrees with measures made using Silviscan (see Chapter Four) which showed only small differences in annual values of MFA existed between treatments. In spite of the small differences, higher velocities were observed in the well watered treatment over the seasonal drought treatments which conforms with results observed by Nanayakkara *et al*. (2014) in one-year-old *P. radiata*.

Measures of velocity were substantially different between this experiment and that of Nanayakkara *et al*. (2014) although the samples tested only differed in age by one year. Resonance based measures of velocity at 12% MC independent of bark and branches in the Nanayakkara *et al*. (2014) study of one-year-old *P. radiata* were approximately 2800 m s^{-1}, while in this experiment of two-year-old trees, they were approximately 4000 m s^{-1}. Whilst the acoustic techniques used in each study differed, diminishing the direct comparability of the results, the large difference in velocities between the Nanayakkara *et al*. (2014) study and this experiment may reflect the rapid improvement in wood properties resulting from increasing cambial age in the corewood zone of *P. radiata*.

The large within tree variation in velocity for the well watered treatment compared to the seasonal drought treatments suggested that the well watered trees had a greater range in MFA. This was not supported by radial measures of Silviscan MFA reported in Chapter Four, however, the two methods were conducted on wood samples at different scales. The well watered treatment did have a greater range in velocity and if the range in MFA of the tested samples in this chapter was large, then this may explain the higher values of shrinkage, which could be due to greater rates of intra-ring differential shrinkage. It was also noted that a weak yet statistically significant correlation between ring width and within tree variation in measures of USV existed implying that increased growth rate was associated with more variable wood formation.

The moderately correlated but significant relationships between USV and Silviscan measures of MFA and *E*, suggest that the USV scanner may be an effective surrogate for obtaining measures of MFA and *E*. The relationships found in this experiment were not as strong as that reported by Sialumba (2011), however, in that study of 16-year-old trees, a much greater range in acoustic velocity, MFA and *E* were observed compared to the relatively narrow range over one growth ring found in this experiment. Further wood properties research may benefit from this relationship as the USV scanner is a fast and cheap to test method compared to the slower, higher resolution and expensive Silviscan method. However, while the low resolution measures of the USV scanner may be suitable for some analysis of wood properties, it is unsuitable for high resolution, intra-annual analysis of wood properties, as it provides a limited number of readings per disk on young two-year-

old material like that of this experiment, and is probably better suited to between ring analysis of wood properties.

Values of tree level longitudinal shrinkage in this experiment were small (0.32 - 0.87%) and narrow in range (0.55%) compared to those found in other studies of young *P. radiata* (Apiolaza *et al*., 2011; Chauhan *et al*., 2013; Nanayakkara *et al*., 2014). Nanayakkara *et al*. (2014) observed a range of approximately 1.4% while Apiolaza *et al*. (2011) observed a range over 2.5%. It is well documented that above a certain MFA threshold (> 30-35 degrees) there is an exponential change in longitudinal shrinkage (Huang and Lambeth, 2007). Given the annual average values of MFA obtained from Silviscan across treatments was 31 degrees, values of MFA in this experiment may have been too small to induce larger values of longitudinal shrinkage as was expected. A weak but significant correlation between longitudinal shrinkage and *E* was observed but no significant correlations were found between shrinkage and MFA, air-dried density and ultrasonic velocity. A strong negative correlation between MFA and velocity has been previously reported (Ivkovic *et al*., 2009; Lachenbruch *et al*., 2010; Hasegawa *et al*., 2011). Given that longitudinal shrinkage decreases with lower microfibril angles (Walker and Butterfield, 1996; Yamashita *et al*., 2009), it was expected that higher velocity (and derived *E*) would have resulted in lower values of shrinkage than was observed in this experiment and previously noted by others (Apiolaza *et al*., 2013; Chauhan *et al*., 2013; Nanayakkara *et al*., 2014). This outcome might have been as a result of the narrow range in values of shrinkage found in this experiment and the greater range in values for MFA, air-dried density, *E* and ultrasonic velocity, which made it difficult to form correlations.

The amount of variability in longitudinal shrinkage between the two samples representing opposite sides of the tree for 14 well watered trees was interesting. In addition to the difficulty of testing small piece size material is the added complication that young *P. radiata* stems seldomly grow completely straight and tend to form compression wood with continuous reorientation (Chauhan *et al*., 2013). The presence of compression wood within the samples may have contributed to the anomaly between the samples on opposite sides of the tree, however, no apparent compression wood was noted in these samples and how it could have been largely isolated to one side of the tree is puzzling given the highly controlled experimental setup. In any case, a large amount of randomly distributed compression wood has been previously reported in juvenile *P. radiata* trees. Values include a range of 6 - 29 % in a three-year-old clonal trial of *P. radiata* in New Zealand (Lindström *et al*., 2004) and 6 - 15 % in four-year-old *P. radiata* trees growing in Chile (Lachenburch *et al*., 2010). Such random distribution of compression wood creates uncertainties in wood property values from young trees, as compression wood substantially differs in its physical and mechanical properties from normal wood (Nanayakkara, 2007). Since longitudinal shrinkage in

compression wood is greater than in normal wood (Harris, 1977; Xu *et al*., 2009), a small amount of mixing or contamination of compression wood in the samples would result in larger values of longitudinal shrinkage in the affected samples. It was also observed that these samples had much lower values of *E* as a result of low velocities, implying high MFA. Given high MFA is characteristic of compression wood (Nanayakkara, 2007), this supports the conclusion that compression wood was present in the affected samples.

CONCLUSIONS

This experiment highlights the effects that the timing and duration of water deficits has on acoustic velocity and longitudinal shrinkage in juvenile *P. radiata.* Acoustic velocity of the well watered treatment was 116 m s^{-1} faster than the nearest water deficit treatment and exhibited a significantly larger within treatment variation in velocity (698 m s^{-1} vs. approximately 474 m s^{-1}). The well watered treatment also exhibited the highest values of longitudinal shrinkage which were at least 8% greater than the water deficit treatments and the largest intra-tree variation in shrinkage. The range in longitudinal shrinkage across all trees (0.32 - 0.87%) was narrow compared to other studies, suggesting water deficits have minimal effect on corewood longitudinal shrinkage. Weak to non-existent relationships were observed between longitudinal shrinkage and other wood properties.

CHAPTER SIX

CONCLUDING REMARKS

Raising the profitability of commercial forestry investments is imperative for the entire New Zealand forestry sector in order to compete with other land users and foreign competitors. Furthermore, the assurance of an internationally competitive, sustainable, consistent raw material will increase investors' confidence to commit into the processing sector. To achieve this, the forest industry wants to better understand the processes limiting productivity by examining environmental physiology in order to understand and quantify the drivers of growth and wood quality.

This study sought to examine patterns of *P. radiata* growth and wood variation within the context of its growth history. By assessing water deficit interactions within juvenile trees and conducting intensive tree growth and wood property sampling, the work contained within was attempting to quantify individual tree responses to water deficits with a view to important characteristics necessary for value maximisation and end product performance. This study differed from others for a number of reasons. This study examined the innermost wood of the troublesome corewood zone of *P. radiata*. The two-year-old trees fall within the most critical phrase of a tree's life (or rotation) as considered by forest managers; the establishment, survival and initial growth stage. Consequently, it is of interest to forest managers to understand the response of young trees to environmental factors that may impact on survival, growth and the scheduling of operations. This study also had a very wide scope. Morphology, physiology, carbon dynamics, wood formation and properties were all examined in order to observe and understand the whole of tree response to water deficits. Lastly, the experimental treatments, in particular, the cyclical drought treatments targeting early- and latewood development were largely novel but also extremely relevant. The effects of well watered and summer drought conditions is reasonably well understood by forest managers, however, the effects of spring and autumn droughts are not. By assessing all four scenarios within the context of one growing season, a unique experiment has been conducted.

In contrast to the vast majority of previously published studies, this study was focused on the timing and duration of water deficits opposed to the relative amounts of water available for tree growth. Given that morphological, physiological and phenological processes can be different between spring and autumn and that different types of wood are being formed (earlywood vs. latewood), it is necessary to examine how early and late season drought compares with well watered and extended summer drought conditions. Specifically, this study sought to (i) assess key morphological and physiological processes influenced by water deficits, (ii) quantify carbon fluxes

and partitioning in response to water deficits, (iii) investigate the effect of water deficits on wood property formation, and (iv) examine the relationship between tree growth and wood property formation.

Seasonal water deficits resulted in large fluctuations in needle water potential, ultimately resulting in substantial reductions in stem dimensions and canopy size as hypothesized. The trees growing under different water deficit treatments were expected to have employed different physiological and morphological strategies to cope with their particular water deficit regime. Reduction in stem diameter growth was more marked for the late season treatment than the early season treatment, when compared with the well watered trees, although the late season treatment experienced lower values of water stress integral. During the imposition of the late season drought treatment, physiological measures showed the extent to which photosynthetic rate, stomatal conductance and transpiration were impacted, resulting in reduced intercellular CO_2 concentration. The lower CO_2 concentration during periods of water deficits was reflected in values of the stable carbon isotope ratio ($\delta^{13}C$), which was most enriched for the summer drought treatment, followed by the cyclical drought treatments, when compared with the well watered treatment.

Needle loss is linked with imposed physiological stress. This was unsurprisingly greatest for the summer drought treatment, but also large for the late season drought treatment, although it experienced lower values of water stress compared with the early season drought treatment. Even though a reduction in branch diameter and length accounted for most of the water deficit induced decrease in crown dimensions and volume, decreased needle length and fascicle numbers also contributed substantially to the reduction. The more marked reductions in stem and crown growth for the late season drought treatment, compared with the early season drought treatment, possibly indicate that tree size had an impact on the ability of the trees to respond to water deficits. The early and late season drought treatments were most often not significantly different from each other, and served as an intermediary between the well watered and summer drought treatments for the vast majority of measures conducted.

A strong effect of water deficits on photosynthesis resulted in large differences in carbon fluxes to various tree components, with treatment values of GPP exhibiting an approximately three-fold variation in carbon. Water deficits significantly decreased fluxes to GPP, NPP, ANPP and TBCF following gradients of tree productivity. In spite of large differences in tree growth, size and carbon fluxes between treatments, partitioning of GPP to ANPP and TBCF was not impacted, suggesting that water deficits are more important in delaying growth than in changing patterns of carbon allocation in juvenile trees. This result disagrees with the second thesis hypothesis. The summer drought treatment was found to have the same ΔC_R /ANPP ratio as the other treatments, which is generally

inconsistent with commonly observed increases in the root to shoot ratio when water availability is limited. Irrespective of previous water status and tree size, woody biomass and foliage biomass were highly correlated across all trees, indicating the very strong functional relationship between conducting tissue of the tree and foliage.

The most interesting element of this study was the within ring analysis of wood properties which demonstrated the highly variable nature of wood formation. Water deficits impacted on rates of photosynthesis and respiration directly reducing growth by decreasing CO_2 assimilation and reducing cell division. The effect of water stress was most evident on cell wall expansion resulting in smaller diameter cells and therefore, a higher cell population for a given area. During periods of water deficits, there were no significant changes in cell wall thickness, although cell wall thickness appeared to be the primary driver of increasing values of density later in the growing season. The impact of increased cell population was a corresponding increase in density and *E*. The initiation of drought conditions produced a rapid transition to latewood tracheids and growth curtailment, however, this effect was not as pronounced for the early season drought trees as the late season and summer drought treatments. Such a transition during periods of water deficits is a physiological process balancing mechanical support requirements and water stress resistance. A major effect of well watered conditions was to produce a more gradual transition from thin walled earlywood tracheids to thick walled latewood tracheids, ultimately resulting in a smoother profile for density and *E* during the year. However, at the same time, the well watered treatment exhibited the greatest intra-annual variation in values of density, cell wall thickness and *E*. It is possible that this would have resulted in higher differential shrinkage within the annual ring of the well watered treatment.

Strong interactions between treatment and time of the year were evident, particularly for density and *E*. While seasonal water deficits often resulted in significantly different values of wood properties during and after imposition of the relevant treatments, absolute variations in density and *E* were small compared to variations exhibited over the course of the growing season. Much greater treatment variations were observed for MFA with treatment induced variations nearly matching annual variation. These results suggest that the annual transition of within ring values of MFA is heavily regulated through water deficits supporting the third hypothesis, however, this does not appear to be the case for density and *E*.

MFA gradually declined over the course of the year for all treatments, but the imposition of water deficits and the release from water stress resulted in changes from the well watered treatment. The early season drought trees showed the least amount of deviation from the well watered trees. The variation in values of MFA when compared to other studies, was small. This is

likely to be the result of two factors: the trees were grown in a polyhouse free of wind induced sway and thus not required to constantly realign themselves; and that the trees were from a high velocity (implying low MFA) clone. In contrast, *E* exhibited the greatest variation amongst the measured wood properties during the growing season for all treatments, increasing three-fold for the well watered treatment from values of 3.2 GPa in spring to 9.85 GPa during early winter.

Strong seasonal variations in wood properties as a result of imposed water deficits did not translate into annual differences between treatments which at the seasonal level agrees with the fourth thesis hypothesis, but at the annual level, rejects it. Variations in wood properties during imposition of water deficits were completely offset by post treatment values of wood properties for density, MFA and *E*. This result may suggest that whilst water deficits are important at the sub-annual level, they are not at the annual level. Previous work by Cown *et al*. (1991), Watt *et al*. (2006) and Watt and Zoric (2010) across nationwide gradients in environment, covering the range in which *P. radiata* is grown, have shown that temperature is considerably more important in determining wood properties than measures of water availability. Further research into water deficits over longer timescales would possibly provide clarification on the relative importance of water deficits on sub-annual and annual wood properties in mature wood. The lack of annual variation in density, MFA and *E* between treatments in this experiment is a positive finding and of interest to forest growers as these results suggest that in the context of this experiment, achieving fast growth in trees is not detrimental to ring level wood properties in juvenile *P. radiata*. Although this result was not explicitly supported by the acoustic velocity results, observations of acoustic velocity demonstrated that well watered, fast growing trees were not adversely affected as a result of high radial growth rates.

Forest growers need to be able to understand the consequences of water deficits on their business. This study demonstrated that water deficits have a greater impact on growth than on partitioning of carbon or ring level wood properties. Seasonal patterns of growth were significantly affected by the timing and duration of water deficits, however, at the annual level, there were no differences between early season and late season drought. At the seasonal level, water deficits also had a significant effect on wood properties, with values of density and *E* increasing and MFA decreasing. This study has provided much needed information on the impact of water deficits on juvenile tree development, however, scaling of these results to mature trees is not possible as different phenological and physiological processes occur in mature trees, plus cambial age and the environment impact on wood formation processes. As such, information on the impacts of water deficits needs to be extended to further age classes and interactions with other environmental variables. Filling the remaining knowledge gaps, both with regards to biological systems

understanding and assessment methodology are crucial for the design of adaptive management strategies and essential to the sustainable and efficient management of forest resources under climate change and forest intensification.

APPENDIX ONE

PRELIMINARY MODELLING OF DENSITY AND MICROFIBRIL ANGLE IN JUVENILE *PINUS RADIATA* IN RESPONSE TO ENVIRONMENTAL VARIABLES

SUMMARY

Modelling of *Pinus radiata* D. Don wood properties in response to environmental variables at the sub-annual level, using high resolution data has not been undertaken before. The objectives of this study were to create models of density and microfibril angle as a function of needle water potential and climate variables to explain variation in wood properties, and to examine the extent to which needle water potential reduced the treatment effect in models of density and microfibril angle. Photosynthetically active irradiance and needle water potential were highly significant climate variables for density and microfibril angle. Temperature was also an important variable for density, but not for microfibril angle. Needle water potential removed or reduced treatment effects when included in the density and microfibril models, respectively. Within ring temporal transition from earlywood to latewood could not be explained solely by the density model and other factors or processes driving that transition may still be unexplained. While this study provided some clarification on the environmental influences on wood properties, the modelling was limited by the lack of needle water potential replication and high levels of multi-collinearity between environmental variables. Further work is required to clarify the environmental causes of within ring density and microfibril angle variation.

INTRODUCTION

Understanding the transition from earlywood to latewood within a growth ring may be an important key to predicting distributions of wood properties within and between trees and sites. Earlywood is characterised by cells with thinner walls, larger lumens, higher microfibril angle (MFA) and lower density compared to wood grown later in a season (Bamber and Burley, 1983; Harris and Cown, 1991; Plomion *et al*., 2001), and it is the amount and nature of latewood that primarily determines important properties such as modulus of elasticity (*E*) and distortion during lumber drying. In practice, wood technologists typically define earlywood and latewood by wood basic density, with earlywood often considered to be wood with basic density below 400 kg m^{-3} in a ring. Clearly though, there is no sharp division between earlywood and latewood, and gradients exist within growth rings. A preliminary study of factors related to the transition between earlywood and latewood was feasible using the data collected during the study reported in this thesis. Both environmental and drought treatment factors were likely to be important in the study, and so both needed to be considered.

Responses of wood properties of *Pinus radiata* D. Don to environmental gradients have been extensively studied at between-ring, between-tree and between-stand levels (e.g. Harris, 1965; Harris *et al*., 1978; Cown and McConchie, 1983; Watt *et al*., 2006; Watt *et al*., 2008; Watt and Zoric, 2010; Nanayakkara *et al*., 2014). These studies reported that temperature was the most important factor in determining wood properties within New Zealand, with properties improving in warmer areas. Harris (1965) and Cown and McConchie (1983) both found significant relationships between basic density and mean annual temperature having surveyed 37 and 250 sites, respectively, throughout New Zealand. In both studies, correlations were greater with outerwood (r^2 = 0.88 and 0.49, respectively) than with corewood (r^2 = 0.32 and 0.31, respectively). Cown and McConchie (1983) also found evidence that rainfall was positively correlated with basic density, with winter rainfall being particularly important. In more recent studies examining four-year-old *P. radiata*, Watt *et al*. (2006) found mean annual air temperature and average fractional available volumetric water content, a measure of the root zone water balance, had highly significant ($P < 0.0001$) relationships with *E* (r^2 = 0.53 and 0.33, respectively). In another nationwide study (Watt *et al*., 2008), average air temperature and basic density were positively and significantly related (r^2 = 0.34; $P = 0.01$), however, it was average minimum air temperature during winter that was included in the final multiple regression model for basic density. In practice, these studies have demonstrated that warmer, wetter sites tend to produce trees with higher than average density.

Although temperature is the dominant driver between sites, experimental studies have shown that water deficits significantly retard tree growth and influence wood properties in juvenile

P. radiata. Harris *et al*. (1978) found that periodic moisture stress applied to container grown trees increased earlywood basic density, mean density and latewood ratio compared with a treatment that had no deficits, although results were not always consistent within annual rings. Fibres were also shorter in trees grown under water stress. Imposed water stress has also been found to reduce measures of acoustic velocity and *E*, have no effect on density but increase longitudinal shrinkage, when compared with a well watered control (Nanayakkara *et al*., 2014).

While such studies have improved our knowledge of the impacts that environmental variables have on wood properties of *P. radiata*, these studies are typically at a low resolution examining annual properties or properties at one point in time. Advances in measurement techniques using automated scanning X-ray diffractometry and X-ray absorption, such as Silviscan (Evans *et al*., 1995; Evans *et al*., 1999; Evans, 2006), allow wood properties to be examined at scales as low as 25 µm, providing extremely high resolution information. Previous high resolution, sub-annual studies examining the effect of climate variables on wood properties have been predominantly in *Eucalyptus* species (e.g. Wimmer *et al*., 2002*a*; Wimmer *et al*., 2002*b*; Drew *et al*., 2009; Drew *et al*., 2011), however, density and fibre properties of other species have also been examined (Bouriaud *et al*., 2005; Jyske *et al*., 2010; van der Maaten *et al*., 2012). Only one study has knowingly examined the indirect effects of water deficits on high resolution wood properties in *P. radiata* (Watt *et al*., 2005). Such studies typically demonstrate how wood properties fluctuate seasonally, irrespective of water deficits, with wood density exhibiting minimum values during spring and maximum values during winter. Microfibril angle is highest in spring, often continuously decreasing to lowest values for the season in mid-winter.

Models that provide information on within-tree and even within-ring variation in wood properties are important for furthering our understanding of the environmental variables driving wood property formation in *P. radiata*. Knowledge of the variables driving wood property variation will allow the industry to predict the practical implications of site and climate variables on wood properties throughout the rotation, and provide researchers with added direction for further wood properties research. Development of models utilising high resolution information has previously been undertaken in *P. sylvestris* for wood density and MFA (Auty *et al*., 2013; Auty *et al*., 2014), while in *P. radiata*, Watt *et al*. (2005) created a model using a combination of linear and non-linear functional forms to test ring width as a predictor variable for density, MFA and *E*. However, development of models examining the influence of climate variables on within-ring wood properties using high resolution data is very rare and no such studies have been previously reported for *P. radiata*.

The aim of this study was to determine which climatic and drought treatment variables most influenced within-ring wood properties of juvenile *P. radiata* using high resolution Silviscan data. The specific objectives were (i) to create models of density and microfibril angle as a function of needle water potential and climate variables to explain variation in wood properties, and (ii) to examine the extent to which needle water potential estimates reduced the treatment effect in models of density and microfibril angle.

METHODS

Data collection

Using previously obtained data from the polyhouse experiment, a new dataset was created that included predawn needle water potential (NWP), climate variables and wood properties by treatment. Climate variables comprised air temperature (TEMP), relative humidity (RH), vapour pressure deficit (VPD) and photosynthetically active irradiance (PAR). Wood properties comprised density and microfibril angle (MFA). Measurements of predawn needle water potential were made throughout the experiment using a pressure chamber following Myers (1988). Three trees per treatment were sampled on each occasion. Measures of NWP were made fortnightly during August and September 2009, and between June and the end of August 2010. Between October 2009 and the end of May 2010, NWP was measured weekly. From each tree, three fascicles were sampled from the youngest well developed foliage. The fascicles were collected before dawn and stored in test tubes on ice until ψ_e was measured, always within one hour of collection. Measures of NWP at each sampling time are treatment level values.

Climatic variables within the polyhouse were measured throughout the duration of the experiment. Variables measured were air temperature, relative humidity and photosynethically active irradiance. These variables were measured using sensors connected to a data logger (HOBO weather station, ONSET Computer Corporation, Bourne, MA, USA). Vapour pressure deficit was calculated from measurements of air temperature and relative humidity (Allen *et al*., 1998). Air temperature fluctuated between -1 and 35 °C with a mean and standard deviation of 14.3 ± 5.9 °C. Vapour pressure deficit fluctuated between 0.01 and 3.89 kPa with a mean and standard deviation of 0.41 ± 0.53 kPa, while the maximum value of photosynthetically active irradiance was 1023 µmol m^{-2} s^{-1} with a mean and standard deviation of 134 ± 234 µmol m^{-2} s^{-1}.

Measurements of diameter (at height of 0.5 m) were taken monthly using electronic calipers. At the conclusion of the experiment all 96 trees were destructively sampled. From each

tree, 30 mm discs were taken at the point of monthly diameter measures. In order to prevent checking and minimise dimensional changes, the water in the disc samples was replaced with 100% ethanol. Four ethanol exchanges were undertaken, with each exchange lasting two days. After the final exchange, the samples were air-dried to constant weight. At this point, a bark to bark slice centred on the pith was sawn from each disc sample and split into two pith to bark radial samples so that the transverse surface was a strip 2 mm wide. Samples were then conditioned to 20 °C and 40% relative humidity. Radial profiles of density at 12% moisture content[7] and microfibril angle were estimated using SilviScan at a resolution of 25 µm for density and 200 µm for microfibril angle (Evans *et al*., 1995; Evans *et al*., 1999; Evans, 2006). The distance from the pith associated with each wood property was converted to a time during the year of the experiment. This was done by directly matching distance from the pith to measures of diameter taken during the experiment and accounting for bark thickness and elliptically shaped trees.

Model development

As NWP was the lowest common denominator, measures of climate variables and wood properties were summarised a week either side of the NWP measurement dates to create a 14 day average of each climate and wood property variable. From this point onwards, reference to climate variables includes NWP. Variables were tested for normality and homogeneity of variance and transformations were made as necessary using scaled power transformations (Equation 1) to meet the underlying statistical assumptions of the models used, primarily that the frequency distributions should be as close to normal as possible. The transformed values for each variable are shown in Table 1. All interactions were made with transformed values if the main effects were transformed.

$$x^{(\lambda)} = \begin{cases} (x^{\lambda}-1)/\lambda & \lambda = 0 \\ \log(x) & \lambda \neq 0 \end{cases} \quad (1)$$

The inverse of a scaled power transformation, which is needed to obtain the value of a transformed dependent variable is shown in Equation 2.

$$Y = \left(\lambda Y^{(\lambda)} + 1\right)^{\frac{1}{\lambda}} \quad (2)$$

Decision tree models were created to assess what variables affected density and microfibril angle and the importance of the variable on the wood property in question. The model is fitted using

[7] All further references to Silviscan density are to density at 12% moisture content (referred to as air-dry density)

binary recursive partitioning, whereby the data are successively split along coordinate axes of the explanatory variables so that, at any node, the split which maximally distinguishes the response variable in the left and right branches is selected. Splitting continues until nodes are pure or the data are too sparse (Crawley, 2007).

Table 1. Transformed values for climate variables.

Variable	λ value
NWP	4.45
TEMP	1.22
RH	0.48
VPD	0.67
PAR	-0.51

Using this information to reduce the number of candidate independent variables, linear models were created using the relevant climate variables to explore which terms and correlations were important. Where vapour pressure deficit was in the model, relative humidity was left out, and vice-versa. This was because vapour pressure deficit is calculated using relative humidity and temperature. However, temperature was not removed as it has important effects on phenological and physiological processes such as metabolic and respiration rates (Landsberg and Waring, 2014). Scatterplot matrices were created to examine the relationship between climate variables and each wood property. It was clear from these matrices that a high degree of multi-collinearity existed between climate variables. As a result, analysis of variance tests were performed between models as terms were removed to assess whether models with variables removed were significantly different from those models without the variables removed. This was done until a suitable final model was created that best explained each wood property.

The Durbin-Watson test was conducted to detect the presence of autocorrelation (Crawley, 2007). As serial autocorrelation was present for both linear models, mixed effects models were created with treatment as a random effect. This was necessary because at any given NWP measurement date, tree dimension and wood property values were available only at the treatment level and so treatments represented the categories for serial correlation. Mixed effects models were created with and without NWP in the model to test the power of the random treatment term in the models by examining the random effect coefficients. Residuals of the model were examined and the Shapiro-Wilk normality test was conducted to test the normality of the residuals.

Effects graphs were created to demonstrate the effects of and interactions between the independent variables on values of density and microfibril angle. The graphs were created taking

into account the multi-collinearity between variables so that relationships were represented only on areas of graphs that actually occurred. The data points in graphs were the two weekly average values for the four treatments for density and microfibril angle, respectively. The relationship lines created within each graph cover the range in variables observed and reflect actual values of the variables.

All analyses were carried out using R software (R Core Team, 2013). Mixed effect models were used that included the climate variables as a fixed effect with treatment as a random effect.

RESULTS

Density

The final mixed effects model for density included all variables, except relative humidity which was excluded based on the density tree model (Figure 1). All terms and the interaction between photosynthetically active irradiance and temperature were highly significant ($P < 0.001$) and the model explained 57% of the variation in density (Table 2). The model residuals (Figure 2) had a normal distribution ($P = 0.1526$). In the model with needle water potential, treatments had the same coefficients, however, in the model without needle water potential, the coefficients were different.

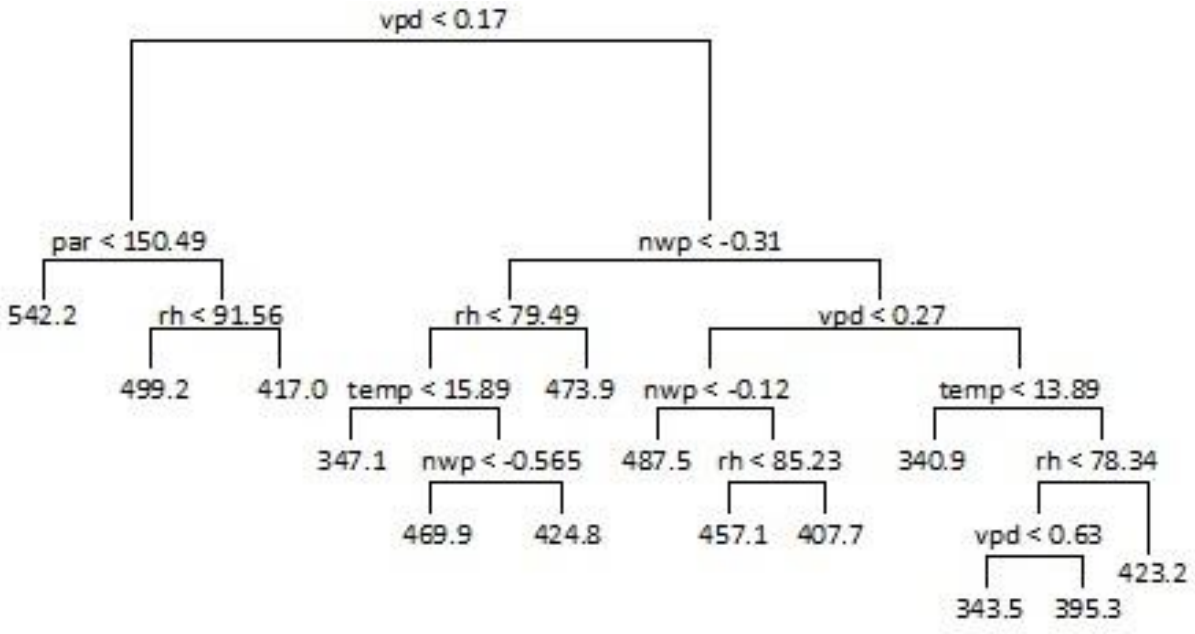


Figure 1. Density decision tree model.

Table 2. Density mixed effects model summary.

Variable	Coefficient	Standard error	P-value
Intercept	2.349	0.022	0.000
VPD	-0.077	0.009	0.000
PAR	0.001	0.000	0.000
NWP	-0.416	0.062	0.000
TEMP	-0.024	0.003	0.000
PAR:TEMP	0.001	0.000	0.000

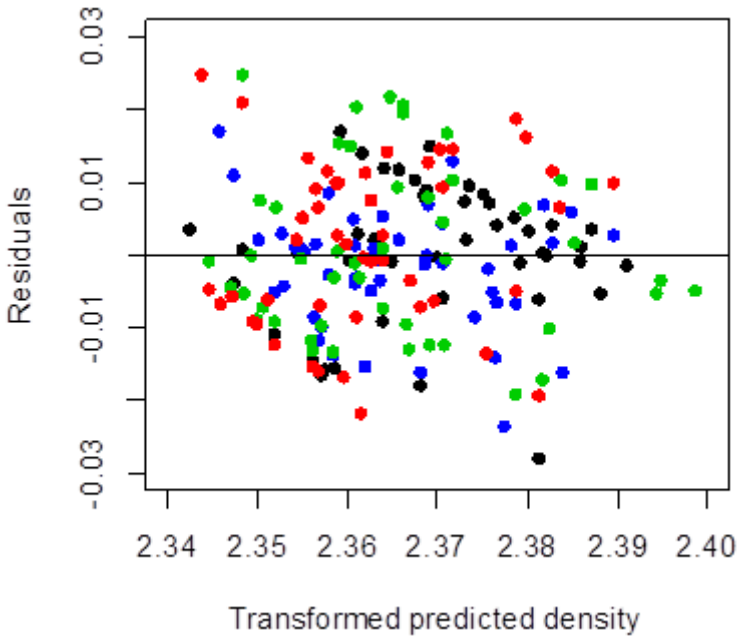


Figure 2. Transformed predicted density vs. residuals for the well watered (red), early season cyclical drought (blue), late season cyclical drought (green) and summer drought (black) trees.

Effects of vapour pressure deficit, photosynthetically active irradiance, temperature and needle water potential on density are shown in Figures 3 and 4. Figure 3 shows a constant decline in vapour pressure deficit as photosynthetically active irradiance and temperature decline. It is at the lowest values for these variables that the highest density values were observed, however, the range in density at all values of vapour pressure deficit, photosynthetically active irradiance and temperature strongly suggests that factors other than the direct impact of environmental variables primarily drive density formation. This graph does however, visually demonstrate the strength of the photosynthetically active irradiance and temperature relationship as seen in Table 2. The lack of a strong relationship between needle water potential and density in Figure 4 suggests that water deficits influenced density formation but did not drive it, and along with Figure 3, suggests and supports Silviscan results, that high values of density do not occur during the brighter, warmer, summer months.

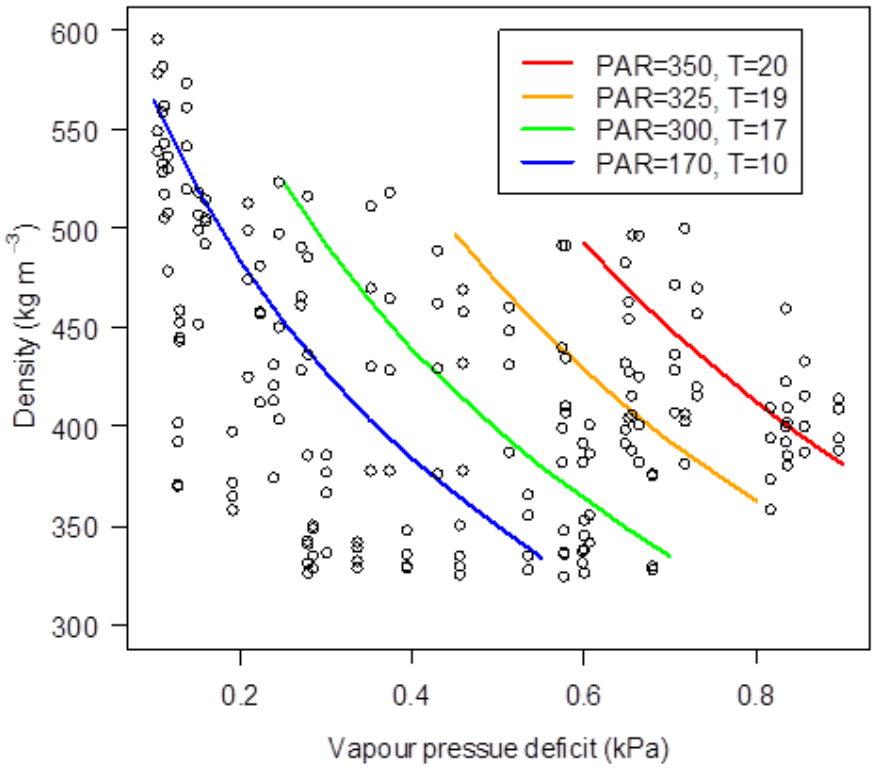


Figure 3. Variation in density with VPD, PAR and temperature. Points show the observed values while the model is shown with coloured lines[8] for different levels of PAR and temperature.

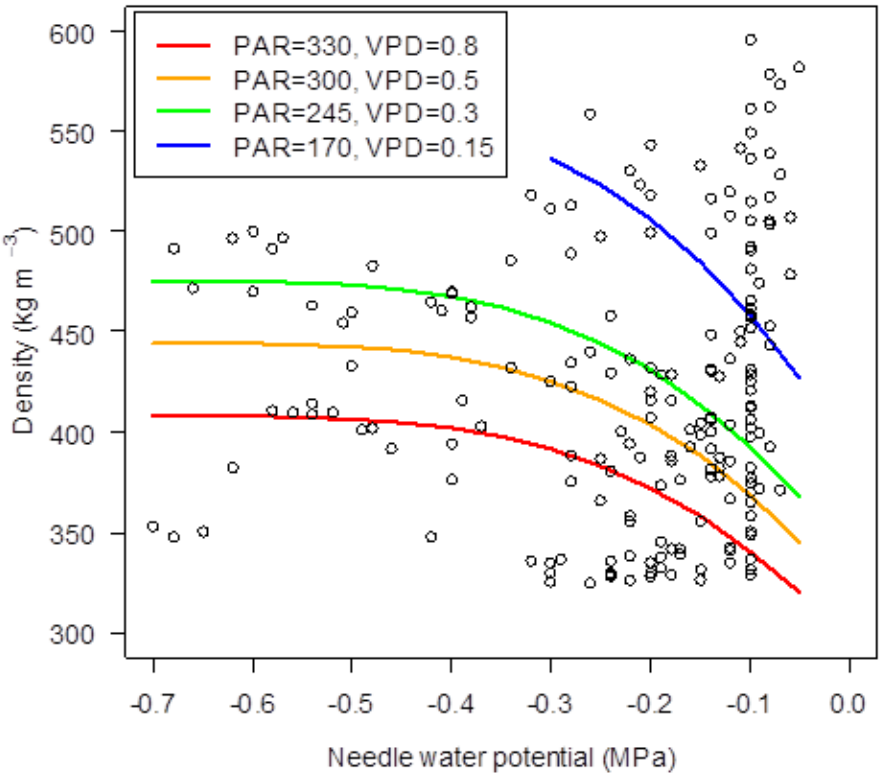


Figure 4. Variation in density with needle water potential, PAR and VPD. Temperature is also a factor in this relationship, however, it is correlated with both PAR and VPD.

[8] This applies to all effects diagrams

Microfibril angle

Temperature and vapour pressure deficit were not included in the microfibril angle mixed effects model based on the decision tree model (Figure 5). All terms that were included in the final mixed effects model were highly significant ($P < 0.001$) (Table 3) and the model explained 50% of the variation in microfibril angle. In the model with needle water potential, the Shapiro-Wilk normality test had a *P* value of 0.02957, as the residuals were slightly skewed towards negative values (Figure 6). When needle water potential was excluded from the model, the residuals had a normal distribution ($P = 0.4442$). Coefficients of treatment were different in the model with and without needle water potential.

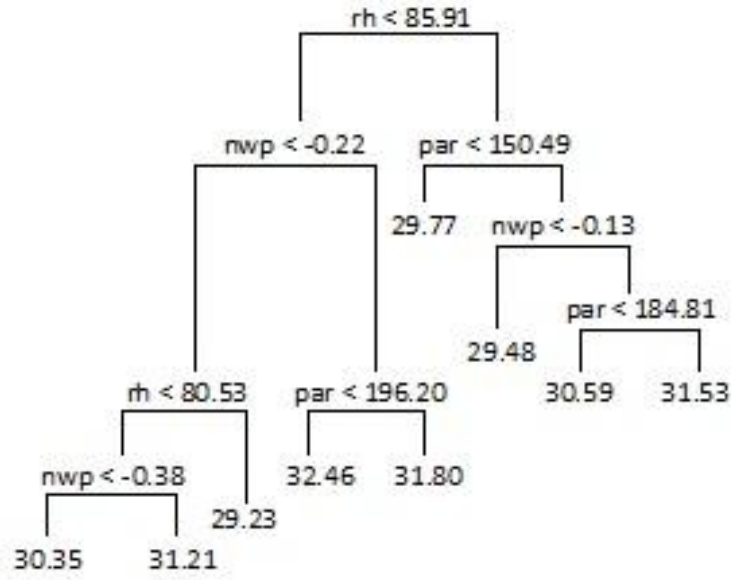


Figure 5. Microfibril angle decision tree model.

Table 3. Microfibril angle mixed effects model summary.

Variable	Coefficient	Standard error	P-value
Intercept	330197.1	53114.5	0.000
PAR	-78.9	19.8	0.000
NWP	1038899.9	300646.7	0.001
RH	-184152.2	30301.9	0.000
PAR:RH	44.8	11.3	0.000
NWP:RH	-584108.5	171698.2	0.001

Different values of photosynthetically active irradiance and temperature had little influence on microfibril angle as relative humidity fluctuated (Figure 7), however, there was a weak general

trend of lower microfibril angles at higher values of relative humidity. A wide range in microfibril angles was observed at needle water potentials of -0.1 kPa, while at higher values of needle water potential, microfibril angle was between approximately 30 and 31 degrees (Figure 8). The relationship between needle water potential, photosynthetically active irradiance and temperature is not clear as lines representing photosynthetically active irradiance and temperature do not follow a high to low gradient and are instead, mixed.

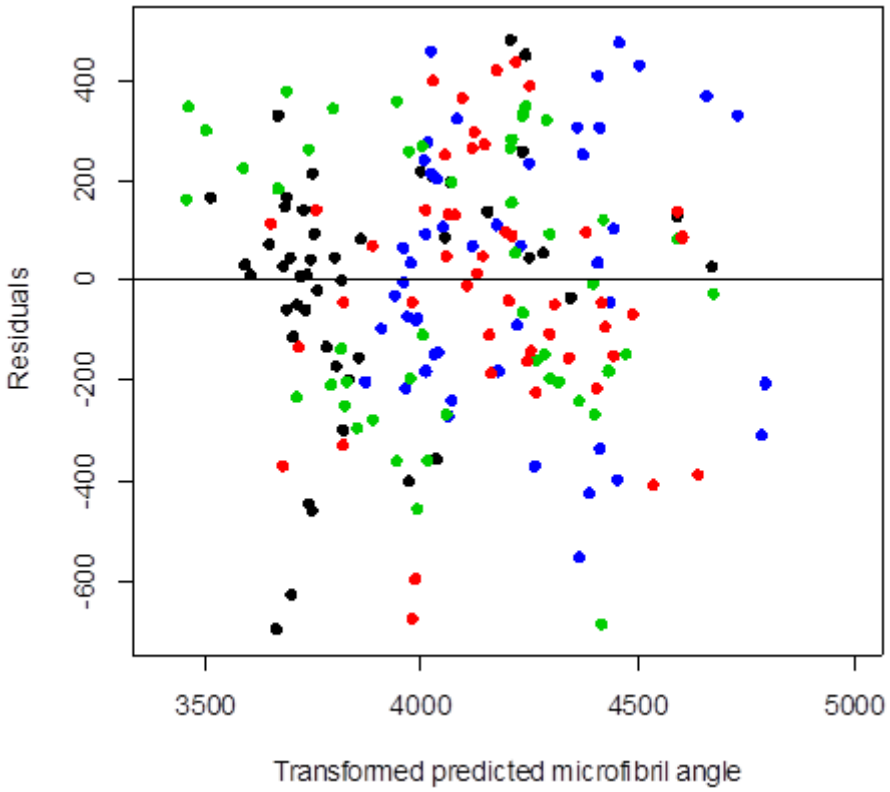


Figure 6. Transformed predicted microfibril angle vs. residuals for the well watered (red), early season cyclical drought (blue), late season cyclical drought (green) and summer drought (black) trees.

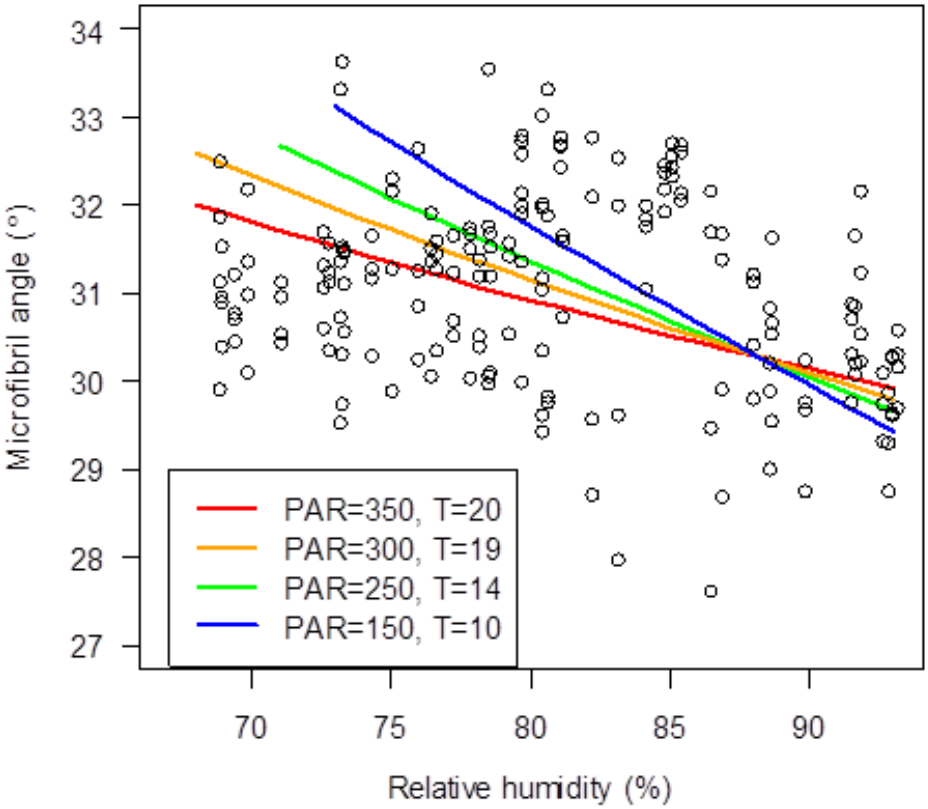


Figure 7. Variation in micorfibril angle with relative humidity, PAR and temperature.

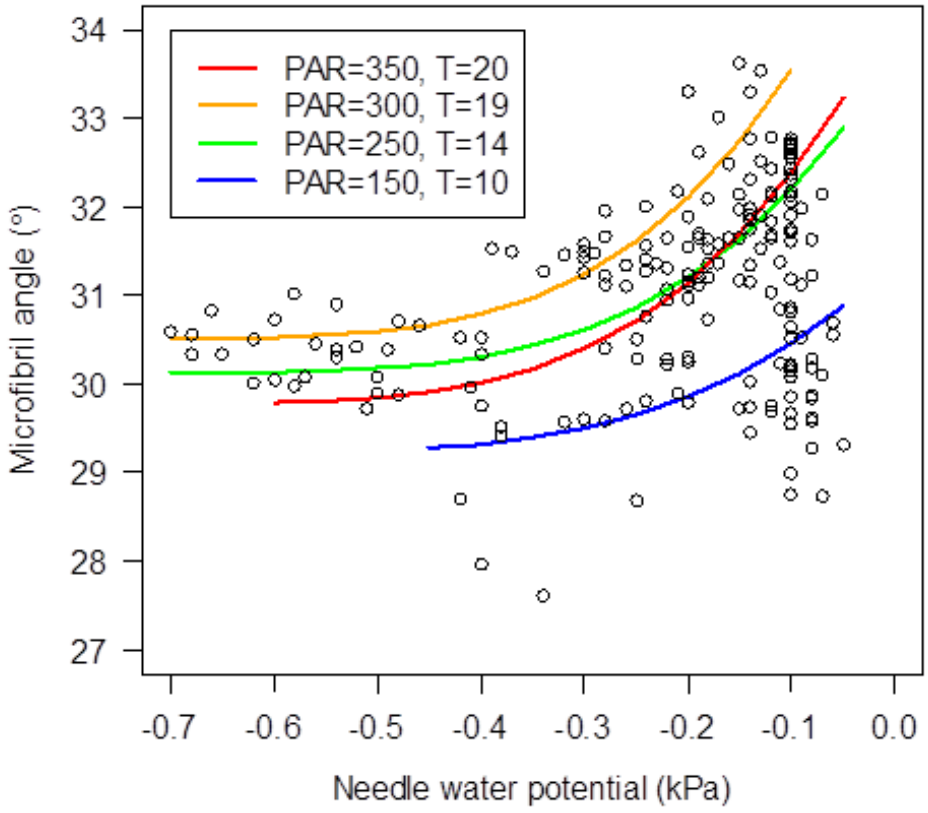


Figure 8. Variation in microfibril angle with needle water potential, PAR and temperature.

DISCUSSION

Final mixed effects models showed that both photosynthetically active irradiance and needle water potential were highly significant climate variables for density and microfibril angle. Values of photosynthetically active irradiance during the course of the year would be generally expected to reflect day length, therefore, it is unsurprising that this variable would be among the most important variables. The effects of photosynthetically active irradiance were most evident with density whereby higher values of photosynthetically active irradiance were associated with lower values of density. An interesting observation was the removal or reduction of the water deficit treatment effects when needle water potential was included in the density and microfibril models, respectively. This suggests that treatment effects were primarily mediated through needle water potential.

Temperature and vapour pressure deficit were highly significant variables for density but not for microfibril angle. Both of these variables may have been mediated through relative humidity for microfibril angle. Previous studies of *P. radiata* have noted the high correlation between temperature and either basic or air-dry density (Cown and McConchie, 1983; Watt *et al*., 2008), while in *Picea abies*, Bouriaud *et al*. (2005) found patterns of increasing density from earlywood to latewood were correlated with temperature, radiation and vapour pressure deficit. In a study of European beech, van der Maaten *et al*. (2012) found that correlation coefficients between mean monthly wood density and monthly climate variables were strongest for vapour pressure deficit and mean air temperature, while radiation and precipitation were weaker. The study reported here largely corroborates these findings, with some minor exceptions.

Given the limitations of the dataset which used treatment level measures of predawn needle water potential, in hindsight, we would have tested predawn needle water potential at the block level to increase the power of the study. The assessment of three trees for needle water potential at each sampling time and the grouping of these results was a serious limitation. Greater replication would have allowed us to have treatment as a fixed effect instead of a random effect. In addition, climate and wood property variables were summarised to 14 day average values as needle water potential was measured either weekly or fortnightly, depending on season, while climate and wood properties were measured at a much higher resolution. Averaging of data may have contributed to the modest explanatory power of the models.

The climate variables displayed high levels of multi-collinearity. This was unavoidable as many climate variables are intrinsically linked and as one fluctuates, others often respond in a predictable manner. A high degree of multi-collinearity existed between temperature, relative humidity and vapour pressure deficit. Vapour pressure deficit is calculated based on measures of temperature and relative humidity (Allen *et al*., 1998). When temperature was included in the

density model, relative humidity was deliberately excluded, however, when temperature was not required for the microfibril angle model, relative humidity was included and was a significant term.

Results of this study are provisional and must be treated with caution given the limitations of the dataset. However, the wider study of tree growth and wood properties within this thesis provided an opportunity to examine what environmental variables were correlated and potentially influencing density and microfibril development. The results provide direction for further work using replicated data and higher frequency sampling for needle water potential.

Lastly, it is relevant to test whether or not the model of density has in any way explained the temporal transition from earlywood to latewood. Plotting the residuals against time (Figure 9), suggests that while the model has explained a significant proportion of the variation in density and has brought the treatments together in the plot, residuals were biased with time. The pattern mostly replicates the commonly observed transition of increasing density across a growing season and so that process remains a mystery.

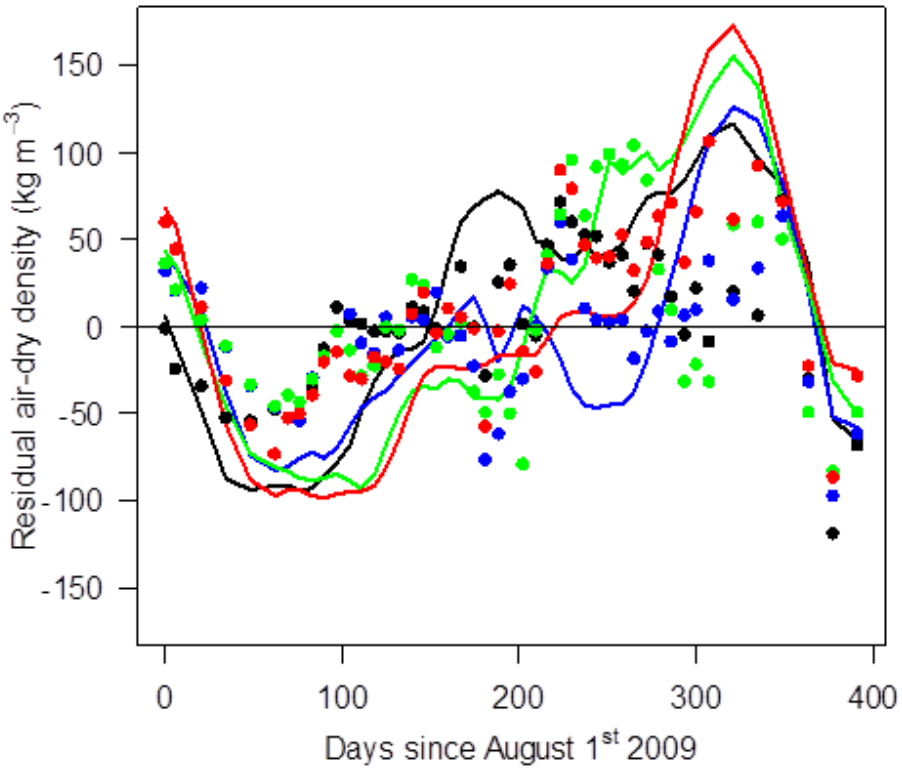


Figure 9. Residuals of the density model versus time for the well watered (red), early season cyclical drought (blue), late season cyclical drought (green) and summer drought trees (black). Lines show the observed value deviations from the overall mean and points show model residuals.

CONCLUSIONS

Modelling of density and microfibril angle variation using climate variables revealed that photosynthetically active irradiance and needle water potential were highly significant climate variables. Vapour pressure deficit and temperature were also significant single terms for density, while relative humidity was highly significant for microfibril angle. Inclusion of needle water potential in models, removed or reduced treatment effects on density and microfibril angle, respectively. Despite explaining some of the variation in wood quality, environmental variables tested in this study did not substantially explain the annual transition from earlywood to latewood.

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